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TOBACCO RESEARCH

Progress Report

A summary of current program and preliminary report of progress of the United States Department of Agriculture and related work of the State Agricultural Experiment Stations.

This progress report is primarily a research tool for use of scientists and administrators in program coordination, development, and evaluation; and for use of advisory committees in program review and development of recommendations for future research programs.

The summaries of research progress include some tentative results that have not been tested sufficiently to justify general release. Such findings, when adequately confirmed, will be released promptly through established channels. Because of this, the report is not intended for publication and should not be referred to in literature citations. Copies are distributed only to members of Department staff, advisory committee members, and others having a special interest in the development of public agricultural research programs.

This report also includes a list of publications reporting results of U.S.D.A. and cooperative research issued during the past year. Current agricultural research findings are also published in the monthly U.S.D.A. publications, Agricultural Research and The Farm Index.

UNITED STATES DEPARTMENT OF AGRICULTURE
Washington, D. C. 20250

December 31, 1968

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CURRENT SERIAL RECORDS

RESEARCH ADVISORY COMMITTEES

The following Research Advisory Committees were established pursuant to Title III of the Research and Marketing Act of 1946:

- | | |
|------------------------------------|--------------------------------|
| 1. Farm Resources & Facilities | 8. Cotton |
| 2. Utilization Research & Develop. | 9. Grain and Forage Crops |
| 3. Human Nutrition & Consumer Use | 10. Horticultural Crops |
| 4. Marketing | 11. Oilseed and Peanut Crops |
| 5. Agricultural Economics | 12. Plant Science & Entomology |
| 6. Forestry | 13. Sugar Crops |
| 7. Animal & Animal Products | 14. Tobacco |

The source materials used by the advisory committees include organizational unit progress reports and subject matter progress reports. The latter contain information which was first reported in the organizational reports and has been assembled for use by commodity committees. The number prefixes shown below refer to advisory committees listed above.

ORGANIZATIONAL UNIT PROGRESS REPORTS

<u>Agricultural Research Service (ARS)</u>	<u>Economic Research Service (ERS)</u>
1 - Agricultural Engineering	5 - Economic Development
1 - Soil & Water Conservation	4, 5 - Marketing Economics
2 - Utilization -- Eastern	5 - Farm Production Economics
2 - Utilization -- Northern	5 - Economic & Statistical Analysis
2 - Utilization -- Southern	5 - Foreign Development & Trade
2 - Utilization -- Western	5 - Foreign Regional Analysis
3 - Human Nutrition	1, 5 - Natural Resource Economics
3 - Consumer & Food Economics	6 - <u>Forest Service - Research (FS)</u>
4 - Market Quality	4, 5 - <u>Farmer Cooperative Service (FCS)</u>
4 - Transportation & Facilities	4, 5 - <u>Statistical Reporting Service (SRS)</u>
7 - Animal Husbandry	
7 - Animal Disease & Parasite	
12 - Crops	
12 - Entomology	

SUBJECT MATTER PROGRESS REPORTS

- 6 - Forestry (other than Forest Service)
- 7 - Animal-Poultry and Products Research Other Than Husbandry, Disease and Parasite
- 8 - Cotton and Cottonseed
- 9 - Grain and Forage Crops
- 10 - Horticultural Crops
- 11 - Oilseed and Peanut Crops
- 13 - Sugar Crops
- 14 - Tobacco

A copy of any of the reports may be requested from Max Hinds, Executive Secretary, Tobacco Research Advisory Committee, Research Program Development and Evaluation Staff, U. S. Department of Agriculture, Washington, D. C. 20250.

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INTRODUCTION

This report deals with research on tobacco. It covers Farm Research; Consumer and Industrial Use Research; and Marketing and Economic Research. It does not include extensive cross-commodity work, much of which is basic in character, which contributes to the solution of not only tobacco problems, but also to the problems of other commodities. Progress on cross-commodity work is found in the organizational unit reports of the several divisions.

Tobacco research is supported by (1) Federal funds appropriated to the research agencies of the U. S. Department of Agriculture, (2) Federal and State funds appropriated to the State Agricultural Experiment Stations and Puerto Rico, and (3) private funds allotted to research carried on in private laboratories or to support of State Station or USDA work.

Research by USDA

Farm Research in the Agricultural Research Service comprises investigations on breeding and genetics, culture, variety evaluation, diseases, insects, and crop harvesting, handling operations and equipment, and curing. It is carried out in the following divisions: Crops, Entomology, and Agricultural Engineering. The work involves 51.6 scientist man-years of scientific effort.

Consumer and Industrial Use Research in the Agricultural Research Service is mainly concerned with the chemistry and biology of tobacco and the smoke therefrom. Much of the research is basic in nature and health oriented but many findings may be of value to industrial problems not related to health. This work is conducted at Eastern Utilization Research and Development Division, Wyndmoor, Pennsylvania, and the University of Kentucky. The work involves 40.5 scientist man-years.

Marketing and Economic Research is done in two services. Marketing research in the Agricultural Research Service deals with the physical and biological aspects of assembly, packaging, transporting, storing, and distribution from the time the product leaves the farm until it reaches the ultimate consumer. Economic research in the Economic Research Service deals with market structure, practices and competition; product quality; margins, costs, and efficiency; economics of farm production; supply and demand; and outlook and situation. The work reported herein is done by the following divisions: Market Quality Research and Transportation and Facilities Research Divisions in ARS, and the Marketing Economics, Farm Production and Economics, and Economics and Statistical Analysis Divisions in ERS. The tobacco research in the marketing and economic research area involves 13.2 scientist man-years, not counting that devoted to farm production economics, which is shown for tobacco, cotton, and peanuts, involving 17.6 SMY's. For comparison, in 1967 it was estimated that the equivalent of 1.0 SMY was devoted to tobacco.

Interrelationships Among Department, State and Private Research

A large part of the Department's research is cooperative with the State Experiment Stations. Many Department employees are located at State Stations and use laboratory and office space close to or furnished by the States. Cooperative work is jointly planned, frequently with representatives of producers or industry participating. The nature of cooperation varies with each study. Ordinarily programs are developed so as to fully utilize personnel and other resources of the cooperators, which frequently includes resources contributed by interested producers or industry.

Research is in progress on all domestic tobacco types in the various production areas with especially close cooperative research at 12 agricultural Experiment Stations. Research effort is devoted to breeding and genetics; diseases, insects, nematode and weed control; quality, culture, physiology, harvesting and curing; and to chemical and physiological studies on health-related aspects of tobacco. These studies are being conducted by the Crops Research, Entomology, Market Quality and Eastern Utilization Research and Development Divisions of ARS. Much of the research is conducted through grants or by contract.

Close cooperation is also maintained with private industry including cigarette and cigar manufacturers, chemical companies, and machinery manufacturers. All of the tobacco companies conduct vigorous and diverse programs designed to improve the quality of the product and reduce manufacturing costs. These companies are also investigating new methods for producing "homogenized tobacco leaf" or "sheet tobacco" for cigarettes or cigar binders or wrappers; developing new tobacco varieties; and determining chemical composition of leaf and smoke. The tobacco companies' work depends considerably upon discoveries resulting from fundamental work by public agencies.

Research by chemical companies is concerned with the development of new tobacco flavoring agents, cigarette paper and filters, chemicals for agro-nomic use, "sheet tobacco" process, and new and improved machinery for manufacturing tobacco products. The Department and other public agencies continue to provide much of the basic data needed to carry out these programs.

The manufacturers of chemicals for disease control and plant growth regulation continue to expand their efforts to produce new products and introduce them into use. The Federal Government assists in this area in the evaluation of new plant growth regulators and their effect on quality.

Basic research done by the Department and States will be utilized by industry and other organizations in their research programs, especially in the further development of improved products and equipment. Industry's cooperation in supporting tobacco research at Federal and State Stations has contributed greatly to its success.

The following results exemplify recent research accomplishments pertaining to tobacco:

Suitability of Tobacco Breeding Lines for Mechanization. Pale yellow an Aurea mutation has the potential for reducing labor costs for harvesting tobacco. Three tobacco introductions possessing the Aurea phenotype, which imparts earliness, and their hybrids were harvested in four or five primings in contrast to the normal six primings for flue-cured tobacco. Inclusion of the factor for earliness in new varieties will provide farmers with an opportunity for producing high yields of tobacco in less time. The possibility of harvesting more leaves in fewer primings suggests this mutation may lend itself to priming by machine. In addition, a number of other breeding lines were subjected to different harvesting methods. These included a conventional six priming control and three harvesting methods in which primings were reduced in number. Yields were reduced slightly as number of primings was reduced. One harvesting treatment in which 1/3 of the leaves were removed in each of three primings showed better performance for agronomic characteristics than the control.

Modifying Cigarette Smoke Composition. As part of the U. S. Department of Agriculture's cooperative program on tobacco and health, the University of Kentucky has been determining the effect of chemicals on modifying smoke composition. Cigarette tobacco is being treated with chemicals such as flame retardants, free-radical scavengers, oxidants and temperature depressants. Some changes in the levels of possible health-related smoke constituents have been detected. Decreases of 27% for total particulate matter (tar); 42% for nicotine; 34% for phenol and 71% for benzo(a)pyrene were obtained. The smoke from the treated tobaccos will also be assayed biologically on laboratory animals to determine if any correlation exists between the decreased levels of the selected smoke components and the tumorigenicity of the smoke. The development of this information will be a major step in selecting additives of practical importance.

I. FARM RESEARCH

 TOBACCO BREEDING, GENETICS, PHYSIOLOGY, CULTURE,
 DISEASES, INSECT CONTROL, AND QUALITY
 Crops Research Division, ARS
USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968 :					Total	
	Research Problem Area				:		
	307	208	207	405			
Maryland (Beltsville)	5.8	1.8	2.4	10.0	:	:	
Georgia	1.4	0.8	0.8	3.0	:	:	
Kentucky	3.9	0.8	0.4	8.2	:	:	
North Carolina	2.4	0.6	0.3	5.3	:	:	
Pennsylvania	0.3	0.5	0.2	0.5	:	:	
South Carolina	0.2	0.8	0.2	1.0	:	:	
Tennessee	2.6	1.3	1.1	5.0	:	:	
Wisconsin	0.5	0.5	0.5	1.0	:	:	
Total Tobacco	17.1	6.6	0.7	9.6	:	34.0	

Intramural program is supplemented by extramural support representing (a) 3.7 SMY's at State Agricultural Experiment Stations as follows^{1/}: Florida Agricultural Experiment Station (0.2 SMY's), Kentucky Agricultural Experiment Station (3.0 SMY's) and North Carolina Agricultural Experiment Station (0.5 SMY's).

1/ RPA 307 = 0.2 SMY's; RPA 405 = 3.0 SMY's; and RPA 208 = 0.5 SMY's.

Problems and Objectives: World consumption of tobacco continues to increase at a substantial rate and our domestic use grows at a much slower pace. At the same time our exports actually are declining, when measured as a percentage of the world market. Concern over smoking and health has an adverse effect on domestic consumption. Production costs make United States leaf less competitive in world trade. The problem is to produce tobacco with a high use value, including smoking and health aspects of quality, and at the same time do it more efficiently. To accomplish this objective a continuous fund of new information is required which will enable the farmer to combat pests, cultivate, harvest and cure good yields of quality leaf at minimum costs.

Special attention is being given to achieving the following objectives:

- (1) Studies to reduce or eliminate any constituents that may be identified as harmful to the user.
- (2) Increased efficiency in harvesting, culture and curing.
- (3) Genetic alteration of tobacco cultivars for mechanization.
- (4) Identification and incorporation of heritable forms of resistance to protect plants from pest damage.
- (5) Studies of growth regulators to modify plant growth.

Progress - USDA and Cooperative Programs

RPA 307 - IMPROVEMENT OF BIOLOGICAL EFFICIENCY

A. Breeding and Genetics

1. Sampling techniques and correlation of alkaloid content in F_2 seedlings with mature plants of the F_5 . Alkaloid determinations were made on green and cured leaves at different stalk positions on field-grown plants and on leaves of seedling at Florence, South Carolina. Significant correlations were obtained between an earlier seedling test of the F_2 and analyses of mature leaves from F_5 plants. High correlations were obtained when the percentage of total alkaloids in green and cured leaves were averaged separately and compared. Analyses also showed that total alkaloid content of top green leaves was closely correlated with total alkaloids in cured leaves. Thus the data indicated that early generation testing of seedlings can give a reliable estimate of the performance of advanced generations and that samples taken from the top of the mature plant can give a reliable estimate of alkaloid levels in cured leaves from the entire plant.

2. Pale yellow, an aurea mutation in tobacco which imparts earliness and may have utility for mechanized harvesting of flue-cured tobacco. An aurea mutation observed in T.I. 1372 and other tobacco introductions is controlled by the dominant gene Py. As Py plants approach maturity all leaves tend to yellow simultaneously. Tests at Oxford, North Carolina, have shown that it is possible to harvest Py tobacco in fewer primings than conventional flue-cured tobacco. In addition, the possibility of harvesting a greater number of leaves in fewer primings suggests that this aurea mutation may lend itself to priming by machinery.

3. Evaluating the suitability of tobacco breeding lines for mechanization. A number of tobacco breeding lines were subjected to four different harvesting methods at Oxford, North Carolina. These included a conventional 6-primings control and three harvesting methods in which the primings were reduced in number. An evaluation of the cured leaf from each treatment indicated that yield was reduced slightly as the number of primings was reduced. However, the dollar value per hundred pounds was not affected by harvesting methods. One harvesting treatment, in which 1/3 of the leaves was removed in each of three primings, showed a better performance for agronomic characteristics than did the control.

4. Cherry-red variegations in flue-cured tobacco. Results obtained at Oxford, North Carolina, indicated that all of the cherry red (CR) segments contained high levels of nornicotine (an undesirable alkaloid) while cherry red-free (CRF) tissues contained principally nicotine. Further, analyses of specific CR segments of leaf indicated that the level of conversion was equivalent to heterozygous tissue C/c, suggesting that the mutational change at the alkaloid locus most frequently involves a single gene. The high frequency of this change suggests that gene "c" is very unstable.

5. Evaluation of divergent strains of tobacco for physical characteristics related to mechanization of flue-cured tobacco. A large number of tobacco introductions (T.I.^s) and their F₁ hybrids with standard flue-cured varieties were examined at Oxford, North Carolina, for agronomic characteristics adapted to mechanization. Three T.I.^s possessed the aurea phenotype which imparts earliness. Most of them, and their hybrids, were harvested completely in 4 or 5 primings in contrast to 6 primings for the flue-cured controls.

6. Comparative performance of burley tobacco hybrids; Burley X Tobacco Introductions (T.I.^s) and their parents. Twelve burley varieties and their hybrids were evaluated in 1967 at Lexington, Kentucky. Comparison of average parental and hybrid performance indicated significant but small amounts of heterosis for six characters. The largest increment of heterosis was 4.77 percent for yield. A diallel analysis of hybrids indicated the presence of considerable variance due to general combining ability and the absence of variance due to specific combining ability. Hybrids between Burley 21 and 25 T.I.^s had an average percent heterosis which ranged from

0.85 for leaf length to 8.15 for total alkaloids. Days to flower, leaf width and percent total alkaloids differed among families.

7. Variation in selfed progeny of doubled haploid stocks of Nicotiana tabacum L. Doubled haploid tobaccos would be useful in genetic and agronomic research if the derived lines were relatively free of genetic changes (isogenic). An evaluation at Lexington, Kentucky, of six, colchicine derived, doubled haploid stocks was made. Except for plant height in one line, the amount of variation among plants of haploid-derived lines was comparable to, or larger than, the amount of variation within conventional inbred varieties.

8. Development of male-sterile Burley 21. Male-sterility is the key to the economic production of hybrid tobacco seeds. This genetic character has been incorporated into Burley 21 at Lexington, Kentucky, a highly important, multiple disease resistant, burley variety.

9. Developing tobacco breeding lines with resistance to frogeye and brown spot. A heritable form of resistance to frogeye has not been identified in Nicotiana tabacum but is present in Nicotiana knightiana and N. repanda. Resistance to brown spot has been identified in N. suaveolens. Considerable success at Beltsville, Maryland, has been obtained in crossing tabacum x knightiana hybrids with various tobacco varieties and tobacco introductions. Crosses with various tobaccos and N. suaveolens, as well as with a suaveolens x tabacum hybrid, have been made.

10. Breeding for disease resistance in burley tobacco. Breeding lines derived from Nicotiana repanda exhibited exceptionally good resistance to black root rot (Thielaviopsis basicola) at Greeneville, Tennessee; some selections had apparent immunity. A breeding line with 100 percent field resistance to fusarium wilt has also been isolated. Two breeding lines, with high resistance to black shank, black root rot, mosaic and wildfire, which resemble Burley 49 but which have higher resistance to fusarium wilt and higher yielding ability, have been selected for intensive quality evaluation. Resistance to Meloidogyne incognita and M. javanica was found among hybrids involving N. nudicaulis and N. repanda.

11. Genetic variation in sterols. Under contract research with the University of Kentucky, Lexington, Kentucky, biochemical analyses for total sterols were conducted on 18 tobacco introductions (T.I.'s) and the F₁ hybrids between the T.I.'s and the isogenic lines Kentucky Iso 1 and Kentucky Iso 4. Total sterol content of the T.I.'s ranged from 0.946 mg sterols per gram of leaf tissue to 2.183 mg sterols per gram. The total sterol content of Ky Iso 1 was 1.600 mg/g and the range in total sterols among the F₁'s was from 1.44 to 2.232 mg/g. For the crosses with Ky Iso 4, which had a sterol content of 1.900 mg/g, the levels ranged from 1.362 to 1.987 mg/g. In general, the total sterol content for the F₁ generation was close to a mid-parent average.

12. Genetic variation of polyphenols in the genus Nicotiana. Fifty-eight Nicotiana species and seven Kentucky Iso lines were analyzed for total sterols, polyphenoloxidase activity, and the number and activity of peroxidase isozymes. Research was conducted under contract at the Kentucky Agricultural Experiment Station, Lexington, Kentucky. A range of .57 to 6.1 was obtained for polyphenol content. The activity of polyphenoloxidase varied more than 100 fold among the Nicotiana species, while the differences between the N. tabacum selections was very narrow. Variation in number and activity of peroxidase isozymes was also observed among the Nicotiana species.

B. Culture and Physiology

1. Interaction of light and temperature on germination of tobacco seed. All tested seed lots required light for germination immediately after harvest. Some lots continued to be light-requiring as time progressed, but others gradually became light-indifferent. One light-requiring and a light-indifferent selection were intercrossed and self-pollinated at Lexington, Kentucky. Self-pollinated seed was light-requiring or light-indifferent, respectively, according to parentage. Seed from reciprocal crossing differed in dark-germinability and from that of either parent. Both parents contributed toward light sensitivity; however, the contribution of the maternal parent was greater than that of the paternal parent.

2. Pretransplant induction of flowering in burley tobacco. At Lexington, Kentucky, short (8-hr) photoperiods plus cool (18-20° C) temperatures caused floral induction; the same photoperiod in conjunction with 30° C temperatures, did not. Long photoperiods (16-hr) in combination with either warm or cool temperatures did not result in premature flowering. Burley 21 plants that did not have floral primordia at the time of transplanting grew at the same rate and flowered at the same time as uninduced controls regardless of the pretransplant environment (long/warm, long/cool or short/warm). Burley plants exposed to 8-hr high intensity photoperiods and those exposed to 12-hr low intensity photoperiods tend to react similarly to cool temperatures.

3. The effect of photoperiod and end-of-day poising of phytochrome on alkaloid content of tobacco leaves and roots. Plants grown under 16-hr photoperiods grew more rapidly and leaf samples had much higher alkaloid content, per gram of fresh weight, than their counterparts grown at Lexington, Kentucky, under 8-hr photoperiods. End-of-day red or far-red radiation after 16-hr photoperiods, as compared with 8-hr photoperiods, had less influence on morphological development and alkaloid content per gram of fresh tissue. Five minutes of far-red radiation at the end of 8-hr days resulted in elongated internodes and thinner, less dense leaves with more nornicotine per gram of fresh tissue. Root growth was greater on plants that received red as compared with far-red radiation at the end of the day.

4. Effect of KMH on sucker control. The potassium salt of maleic hydrazide (KMH) with a new surfactant was compared with the diethylamine salt of maleic hydrazide (MH-30) at Whiteville, North Carolina. MH-30 and new KMH formulations were applied at the rate of 100, 125, and 150 mg of MH per plant. Percent sucker control increased with increasing amounts of the active ingredient and was greater with KMH than with MH-30 at comparable rates. Sucker control was increased when the level of surfactant was increased in the KMH formulation. Both yield and value of the chemically suckered tobacco were higher than the hand-suckered controls. No differences were noted for percent total alkaloids or for percent reducing sugars among the chemical treatments. The increase in sugars usually associated with MH treatments was evident when compared to the control.

5. Effects of 2, 4-D induced senescence. Plants were sprayed with 10, 30, 70 and 100 mg of 2,4-dichlorophenoxyacetic acid (2,4-D) at topping time to hasten "ripening" at Oxford, North Carolina. Epinasty occurred within 24 hours. Loss of chlorophyll was evident in 5 to 12 days, depending upon the concentration used. Yield was reduced by 20 percent. Plant residues of 2,4-D varied from 3 to 6 ppm up to 53 to 60 ppm for 10 and 100 mg per plant, respectively. Sugars decreased and nicotine, nitrogen, filling capacity, ash and total volatile bases increased. Aroma of the tobacco was abnormal and smoke taste was bitter.

6. Chemically topping Maryland type tobacco. 1-Decanol (T-87) was found to be an efficient chemical topping agent at Upper Marlboro, Maryland. Although hand topping and suckering practices produce slightly better tobacco, chemical topping significantly increased yields but did not significantly decrease price per pound. Chemical topping and suckering agents can aid economical tobacco production; however, applications of the chemical topping agent must be made at the early button stage.

7. 1967 regional tobacco sucker control tests. A variety of sucker control chemicals have been under study throughout the tobacco-producing regions. T-148 (1-octanol and 1-decanol mixture) controlled suckers on all tobacco types in regional tests and was the most effective contact agent on burley tobacco. T-148 averaged 94 percent control and MH-30 (diethylamine salt of maleic hydrazide) and KMH (potassium salt of maleic hydrazide) reduced sucker growth 98 and 94 percent respectively. T-43 (methyl decanoate) was not as effective as T-148 and T-87 (1-decanol). Highest burley yields were obtained with KMH, MH-30, T-148, and T-39S (also a 1-octanol and 1-decanol mixture). Both MH-30 and KMH were found to be effective on cigar tobacco produced in Wisconsin and Pennsylvania. Contact sucker control agents, especially T-148, were very effective and gave 94-96 percent control. T-87, T-43 and Penar (dimethyldodecylamine acetate) gave about 80 percent control at Landisville, Pa. All treatments were effective on dark tobacco with virtually no leaf injury. All of the chemicals also gave practical sucker control on Maryland tobacco.

8. Influence of nitrogen supply on undesirable colors in flue-cured tobacco with particular reference to "toadyness". Toadyness is a term to describe muddy, dull, leaf discoloration. When present to any appreciable degree in cured leaf it results in price reductions. Predisposition to this condition is believed to be inherited and its manifestation the result of unknown factors. A known toady variety, DB 27, and a non-toady variety, NC 2326, were subjected to varying supplies of nitrogen, and grown at different spacings in the field at Oxford, North Carolina. Effects of varying leaf population (a form of nitrogen control) and varying nitrogen levels showed that a diminished supply of nitrogen produced more toady leaf on the susceptible variety, DB 27. The resistant variety, NC 2326, produced little or no toady leaf but expressed nitrogen deficiency in the form of slickness and lack of grain. Early generation screening for predisposition toward toadyness may be accomplished by growing plants under conditions of reduced nitrogen nutrition.

9. Isolation and purification peroxidase enzymes from tobacco tissue. Crude protein preparations resolved into more than six peroxidase isozymes at Beltsville, two of which utilized IAA (indoleacetic acid) as a substrate. Tissues treated with IAA and subsequently extracted for peroxidase enzymes, yielded greater amounts of IAA oxidizing peroxidase enzymes. These results suggest that IAA oxidizing peroxidase is an inducible enzyme.

10. Effects of topping and age of leaves on growth of axillary branches (suckers). Axillary branches (suckers) develop when the apical meristem is removed (topped). Work at Beltsville, Maryland, showed that subsequent development of the sucker is influenced by the age of leaves present on the plant. Young, expanding leaves inhibit sucker growth completely, even after plants are topped. If young leaves are removed, suckers grew despite the presence of an apical meristem.

11. Growth inhibiting substances from Nicotiana tabacum. Young leaves of flue-cured tobacco contain a growth inhibiting substance (s) isolated at Tifton, Georgia. The compound (s) is neutral, of apparent high stability, and the Rf properties on thin-layer chromatograms and column chromatography are consistent. At present, the compound(s) does not appear to have any of the characteristics of previously isolated inhibitors (of the phenolic group) from tobacco.

12. Effects of nitrogen nutrition on the nitrogenous constituents of burley tobacco. Field tests at Lexington, Kentucky, indicate that $\text{NO}_3\text{-N}$, total N, total alkaloids and mineral element composition of field grown burley tobacco is amenable to agronomic control. A high nornicotine tobacco, high rates of nitrogen fertilization and the use of special chemicals high in amine content were used to produce high amine levels in the cured leaf. Leaf is being analyzed for nitrate and secondary amines. Cured leaf will be used for manufacture of experimental lots of cigarettes to determine nitrosamine content, if any, in the smoke.

13. Enzymatic effects on metabolic pathways. Metabolic processes of certain compounds including phenolics, sterols, nitrogenous bases, and isoprenoids in tobacco plants are being studied from green to post-harvest stages at Lexington, Kentucky. The quality and quantity of light affects levels of rutin and chlorogenic acid formation. Also under study are the effects of sterols on permeability of cytoplasmic membranes. In tobacco plants it was found that, in general, total sterols decreased as nitrogen fertilizer levels increased.

RPA 208 - CONTROL OF DISEASES

A. Disease Control

1. Stem resistance to *Phytophthora parasitica* var. *Nicotianae* in tobacco derived from *Nicotiana longiflora* and *N. plumbaginifolia*. Tobacco breeding lines with black shank resistance derived from *N. longiflora* and *N. plumbaginifolia* did not wilt when stems were inoculated with race 0 of *Phytophthora parasitica* var. *nicotianae* at Lexington, Kentucky. In contrast, plants of susceptible varieties or varieties having Florida 301 type resistance wilted and died when stems were inoculated. Stems of all lines and varieties tested were susceptible to race 1 isolates. Even though plants having stem resistance to race 0 isolates were not killed, the fungus was able to make limited growth above and below the point of inoculation.
2. Reaction of *Nicotiana plumbaginifolia*, *N. longiflora*, 18 and Ga 2019-1 to race 1 isolates of *Phytophthora parasitica* var. *nicotianae*. A disease test, using a small plant technique, was carried out with 13 isolates of race 1 at Lexington, Kentucky. Breeding lines Ga2019-1 and L8 were completely susceptible while seedlings of *N. plumbaginifolia* showed a very low level of resistance to black shank. Plants of *N. longiflora*, on the other hand were highly resistant to each of the fungus isolates.
3. Effect of brown spot (*Alternaria tenuis* Nees) infection on certain chemical and physical characteristics of flue-cured tobacco. A typical brown spot lesion on flue-cured tobacco consists of a central necrotic portion, surrounded by a chlorotic "halo" and a non-distinct "pre-halo" zone beyond which healthy tissue is located. Experiments conducted at Oxford, North Carolina, showed the fungus hyphae were confined to necrotic tissue. Changes in the halo and pre-halo zones were the result of some diffusible fungus metabolite. Chemical determinations showed that moisture holding capacity and percent total alkaloids are reduced by about one-half and percent water soluble sugars are reduced 10-fold in lesion-tissue as compared with healthy tissue. No significant differences in total nitrogen were found when the four types of tissue were compared. The N/Nic ratio of lesion tissue was twice that of healthy tissue and the sugar/N ratio was c.a. one-fourth.

4. Resistance to brown-spot in flue-cured tobacco. Two types of resistance to brown spot caused by Alternaria tenuis Nees., were defined in field studies at Oxford, North Carolina. Six flue-cured varieties were tested. PD 121, a cigar type, with resistance from Beinhart 1000-1, showed only 13 lesions per leaf as compared with 334 lesions per leaf on sensitive Coker 298. The flue-cured breeding line NC 8038-5-9 was moderately resistant to infection with 136 lesions per leaf, but exhibited a form of tolerance by inhibiting chlorotic halo formation which is characteristic of highly susceptible varieties. Disease index (number x size of lesions) per leaf ranged from 4.0 for PD 121 to 63.1 for Coker 298.

5. Field inoculation trials of tobacco with lesion nematodes and nematocidal effects of "Temik". (2-methyl-2-(methylthio) propionaldehyde) (0-methyl carbamoly) oxime. Root-knot nematode resistant breeding lines were planted in lesion nematode infested soil at Florence, South Carolina. Pre-plant treatments of soil included applications of a systemic insecticide, Temik. Early plant growth was stimulated by nematode inoculations as indicated by slight increases in yield and acre value. Three breeding lines, however, were depressed by inoculation. Examination of roots showed visible damage from nematode feeding activity. Attempts to recover nematodes from Temik treated plots were not successful. Apparently, the systemic insecticide Temik served to prevent nematode reproduction.

6. An apparent new pathogenic race of Meloidogyne incognita acrita. Tobacco variety NC 95 possesses a high degree of resistance to root-knot nematodes. Since its release in 1961, it has been considered to be genetically stable for resistance. However, during the seasons 1964-66, galling was found on NC 95 in breeding plots at Florence, South Carolina. When grown at other locations, NC 95 remained free of galls. Morphological and cytological studies of female specimens of the root-knot nematodes attacking NC 95 indicated that they did not differ morphologically from other collections of M. incognita acrita. In the absence of morphological differences between the root-knot isolates, the race pathogenic to NC 95 should be considered a physiological variant of M. incognita acrita.

7. 1967 field trials with nematocides for control of root knot and root lesion nematodes. Field trials conducted at Florence, South Carolina, showed that 5 new nematocides provided yield increases of from 92 to 404 lbs per acre as compared with the controls. New products which were superior to DD or Telone (dichloropropane plus dichloropropene) were Temik (2-methyl-2-(methylthio) propionaldehyde--0-(methyl carbamoyl) oxime), Mocap (0-ethyl S, S-dipropyl phosphorodithioate), Penphene (tetrachlorothiophene) and Bayer 25141 (0,0-diethyl, 0-2-pyrazinyl phosphorothioate). Application methods indicated that preplant soil incorporation with granular materials such as Temik, 25141 and Mocap was more effective than post-plant side dress applications. Lesion nematodes were as effectively controlled by Temik as by DD and Telone. Other new nematocides which were equal to

the standards in control of root knot included Thimet-Zinophos (0,0-diethyl, 0-2pyrazinyl phosphorothioate), Niagara 10242 (2,3-dihydro-2,2-dimethyl, 7-benzofuranyl, N-methyl carbamate) and TH 336N (4-chloropyridine hydrochloride).

8. Greenhouse inoculation trials with lesion nematodes (*Pratylenchus brachyurus*) on tobacco breeding lines and *Nicotiana* species. Most *Nicotiana* species and tobacco varieties when inoculated with root lesion nematodes at Florence, South Carolina, were severely stunted. Some *Nicotiana* species, however, such as eastii and sanderae, showed evidence of growth stimulation in the presence of the nematodes. A range in variability of stunting of 7 to 55 percent among varieties and 5 to 41 percent among *Nicotiana* species provided presumptive evidence of resistance. Varieties least stunted were Speight G29 (7 percent) and Coker 139 (17 percent).

9. Spray applications of salicylates and carbamates for control of blue mold in tobacco beds. Repeated use of a spray mixture containing 8-ounces of maneb fungicide (Manganese ethylene bis dithiocarbamate) plus 3-ounces of sodium salicylate in 100 gallons of water has given commercial control of blue mold when applied twice-weekly preceding and during severe blue mold attacks at Tifton, Georgia. Substitution of powdered salicylic acid for sodium salicylate was also highly effective but the acid required the use of a wetting agent. The salicylates were used successfully only in spray mixtures with such carbamates as ferbam (Ferric dimethyl dithiocarbamate), zineb (zinc ethylene bis dithiocarbamate), and maneb.

10. Effect of insect control on the occurrence of virus diseases in Burley tobacco. Field grown burley plants were enclosed in cages made of fiberglass screening to exclude aphids and other flying insects at Greeneville, Tennessee. Of 28 plants, so caged, 26 remained free of virus infection. Uncaged control plants were 90 percent infected. Only potato virus Y was identified as the viral agent in infected plants.

11. Development of a weather fleck type of injury on Havana 503. Havana 503 was planted on 35 percent of the Wisconsin tobacco acreage in 1967 and a physiological leaf spot was of some concern to growers. A similar leaf spotting was noticed on Havana 503 early in its development. Preliminary laboratory tests at Madison, Wisconsin, and Beltsville, Maryland, indicated that Havana 142 and Havana 307 are more susceptible to ozone injury than Havana 503. This suggests that physiological spotting in Havana 503 may be due to inherent predisposition to specific environmental conditions.

12. In-vitro production of a tobacco leaf chlorosis inducing substance by *Alternaria tenuis*. When filtrates of *A. tenuis* were obtained by micro-filtration, drops applied to minute wounds on tobacco caused yellowing of the leaf around the point of application within 24 hours in research conducted at Beltsville, Maryland. Non-inoculated, filtered extracts of media were ineffective in producing chlorosis. Intensity of the yellowed areas was

maximized by increasing glucose in the media to 70 gm/liter, incubation of the inoculated media at 28° C, and incubating treated plants at 28° C under constant light.

13. Effects of air pollutants on selections of tobacco and Nicotiana species used for bioassay of viruses. Certain Nicotiana species and tobacco selections are regularly used for bioassay and indexing of virus strains. When indicator plants are damaged by air pollutants they are unsuitable for virus research. Research conducted at Beltsville, Maryland, showed that exposure of 4 tobaccos and 2 Nicotiana species to 6 levels of ozone fumigation showed that N. rustica var. brasilia and N. glutinosa were most severely injured. Tobacco varieties Ky 35, Samsoun and Xanthi were intermediate in their response, and the Samsoun selection NN was the most resistant to ozone. Treatment with sulphur dioxide showed that N. rustica was heavily injured while injury to N. glutinosa and the tobacco selections was about half as severe. Treatments which combined ozone and sulphur dioxide induced symptoms similar to those produced by ozone alone.

14. Susceptibility of flue-cured varieties to weather fleck and ozone fumigation. Twenty-three varieties were grown under irrigation in a field test at Beltsville, Maryland, and the same entries were grown in a greenhouse supplied with ambient air passed through evaporative coolers. Comparisons of field and greenhouse plants and data from a previous year showed good injury correlations among some varieties but poor correlations among others. Varieties Va. 230, Speight G-7, Speight G-36 and White Gold were the most sensitive varieties under all conditions while Delhi 61, NC 2512, Reams 64, Speight G-3 and Coker 316 were the most resistant. Hicks, Coker 319, McNair 20 and 30, and Coker 187 were flecked extensively in the field, but showed better than average resistance to fumigation. Va. 115 was resistant to fleck under field conditions but seemed to be more sensitive to fumigation.

15. Maryland type tobacco with resistance to tobacco mosaic virus, fusarium wilt and black root rot. After many seasons of testing and selection, a Maryland tobacco line similar to variety Catterton, with resistance to tobacco mosaic virus has been developed at Upper Marlboro, Maryland. This breeding line also possesses moderate resistance to fusarium wilt and black root rot and has excellent agronomic characteristics.

16. Inheritance of resistance to individual isolates of Fusarium oxysporum var. nicotianae in Burley tobacco. To study the inheritance of fusarium wilt resistance, five burley varieties were intercrossed in a diallel arrangement at Greeneville, Tenn., and were tested against four separate isolates of F. oxysporum var. nicotianae. Resistance was predominantly additive, although dominance and epistasis were also important in some genotype-isolate combinations. The mode of inheritance and the genetic effects appeared identical for each isolate. Quantitative inheritance of resistance plus differences in pathogenicity of the isolates suggest the desirability of using a mixture of isolates when screening for resistance.

17. Control of black root rot. The use of chemicals reduced the incidence of black root rot and increased performance of tobacco varieties which had low levels of heritable resistance in North Carolina. However, the level of control imparted by chemotherapeutants was not adequate when black root rot susceptible tobacco was grown in infested soil. Studies on the use of orchardgrass in rotations showed that such rotations reduced the incidence of black root rot appreciably, where either resistant or susceptible tobacco was grown. Best control of black root rot was obtained by the combined use of resistant varieties, chemicals and appropriate rotations.

18. Influence of root lesion nematodes on development of black shank. Contract research conducted at the North Carolina Agricultural Experiment Station revealed that inoculation with root lesion nematodes 1-week before and at the time of artificial inoculation with the black shank fungus caused a rapid development of black shank symptoms and an increased severity of the disease in susceptible varieties. The nematode probably acted as a wounding agent and thereby promoted fungus invasion. In those treatments where the nematodes were added three weeks before inoculation, the symptoms were appreciably delayed. Neither nematode infection nor mechanical wounding were instrumental in altering the black shank resistance of varieties with heritable forms of resistance.

RPA 207 - CONTROL OF INSECT PESTS

1. A search for heritable resistance to the horn and bud worm. Systematic screening of a vast array of germplasm was initiated at Oxford, North Carolina, with the view of isolating and characterizing heritable forms of resistance to these tobacco pests. Over 1000 different forms of cultivated tobacco and 60 Nicotiana species are included. Early results indicate that Nicotiana gossei, a distant relative of cultivated tobacco, is resistant to the feeding damage of the horn worm.

RPA 405 - PRODUCTION OF FIELD CROPS WITH IMPROVED CONSUMER ACCEPTABILITY

1. Variation in the activity of certain hydrolytic enzymes during the curing of Burley tobacco. Hydrolytic activities of protease, acid phosphatase, ribonuclease and deoxyribonuclease enzymes were determined at Lexington, Kentucky, in Burley leaves at different stalk positions during air-curing. A slight decrease in activity was observed during the first 24 hours, a slight increase during the second day, and the level remained relatively constant through the fourth day of curing. The activities then dropped rapidly until the seventh day at which time they were about 20 to 40 percent of the original. There was a very slow decrease from the seventh to twenty-third day of curing. Exceptions to the above patterns of activity-change were noted with protease, in which the activity increased during early curing and decreased after the fifth day.

2. Quantitative changes in fatty acid composition of mature tobacco leaves during the flue-curing process. Long-chain fatty acids, suspected as precursors of carcinogenic compounds in tobacco smoke, were studied at Beltsville, Maryland, as their methyl ester derivatives from the flue-cured varieties Coker 319 and Virginia Bright. Total fatty acid content reaches a maximum about four weeks after transplanting and declines thereafter. A loss of between 40-50 percent was observed during the early stage of flue-curing. During curing the shorter-chain acids, 14:0 and 15:1 increased while 16:1, present as a trace in green samples, disappeared. Long-chain acids, 16:0, 18:0, 18:1, and 18:2 decreased proportionately to the total decline of fatty acids with maturation and senescence. Loss of 18:3, however, occurred at a relatively higher rate.

3. Oxylate metabolism in tobacco and its relation to calcium nutrition. Development of calcium deficiency symptoms in tobacco is associated with the temperature at which the plants are grown. Temperature effects upon the activity of glycolate oxidase have been extended to the varieties Ky 12 and Ky Iso 1. All varieties studied at Lexington, Kentucky, showed the same relationship to temperature as indicated for the initial experiments.

4. Agronomic factors affecting Po^{210} and Pb^{210} content of tobacco. Tobacco seedlings accumulated radioelements Pb^{210} and Po^{210} in their tissues at much higher concentrations than amounts at ambient soil levels in experiments at Beltsville, Maryland. As the plants grow, the levels of these radioelements decrease primarily as a result of dilution. Pb^{210} and Po^{210} content in tobacco seeds is much lower than in leaves of the same plant. Two methods of flue-curing tobacco, a closed system using wood as a fuel, or an open system using kerosene, did not result in significant differences in levels of Pb^{210} or Po^{210} in the cured leaf.

5. Effect of farm production practices on nicotine and total particulate matter (TPM) in cigarette smoke. Nicotine content in tobacco smoke seems largely dependent on nicotine content of the leaf, but TPM of the smoke seems to be more closely related to the amount of tobacco required to make the cigarette, as well as the density and total number of puffs. For example, studies at Beltsville, Maryland, showed that among the six flue-cured varieties tested, the nicotine content in cigarette smoke ranged from 1.3 to 3.0 mg; the TPM content ranged from 27 to 34 mg. In addition, rate of nitrogen fertilization, sucker control materials, stalk positions, degree of leaf maturity, and method of curing affected nicotine and TPM levels.

6. Benzyladenine (BA) activation of axillary buds of tobacco. Buds normally quiescent on intact plants can be activated with the cell division factor - benzyladenine. The kinetics of BA activation of DNA synthesis and the rates of H^3 and C^{14} thymidine incorporation into DNA has been studied at Beltsville, Maryland, at 2, 4, 6 and 24 hours after treatment. Chloramphenicol inhibits bud growth but does not inhibit the incorporation of labeled thymidine into DNA. Variations due to differences within plants, bud position, time of day, storage of harvested samples, and perhaps

endogenous rhythms were observed in earlier studies. Experimental procedures were refined during the past year and no difference was noted in the rate of incorporation between BA treatments and controls at 2, 4 and 6 hours after activation. However, at 24 hours, BA activation is very pronounced.

7. Development of a tissue culture bioassay system for the study of growth regulators. Cell suspension systems are ideally suited for the study of growth-regulating compounds in tobacco because treatments can be made under well-defined chemical conditions. Seven types of tobacco have been grown at Beltsville, Maryland, on chemically defined agar media. In an earlier investigation, N. suaveolens was used to test the effects of growth promoters on quiescent and active buds and a hybrid, F₁ (N. suaveolens x N. langsdorffii), was used to check for the effects of growth inhibitors. However, for the work with cell suspensions, an albino mutant which is lethal as a seedling but which grows well on agar media is being used. Tissue cultures of the albino mutant respond well to benzyladenine when grown in suspension cultures in the presence of 2,4-D. The mutant tissue is sensitive to light but grows well in darkness.

8. Hydroxycinnamic acid amides in various tobacco tissues. Significant amounts of two unidentified phenols were found at Beltsville, Maryland, in floral primordia of the tobacco variety Catterton and genetic tumors which form on the interspecific hybrid, F₁ (N. suaveolens x N. langsdorffii). The unknowns were compared to the amount of chlorogenic acid, which is the major polyphenol usually found in these tissues. The phenolic portion of the molecule has been determined to be 2,4,5-trihydroxycinnamic acid. The non-phenolic moiety appears to consist of at least one or more amino acids linked to the phenolic acid through an amide bond. Evidence obtained thus far indicates that glycine, in different amounts, is present in the two compounds. Attempts have been made to identify enzymes which can split the phenolic from the non-phenolic components of the molecule. A plant lipase (steapsin) can hydrolyze the bond. Emulsin, hemicellulase and cellulase were found to be ineffective.

9. Total sterol content of Burley 21 tobacco. Field-grown Burley 21 tobacco plants were treated with the growth regulator chemicals MH-30 (maleic hydrazide) and Penar (dimethyldodecylamine acetate) at Lexington, Ky. At harvest the tobacco leaves were separated into three parts; bottom, middle and top of the plant (approximately 6-7 leaves per part). The total sterol content of green leaves was higher in the bottom parts and lower in the middle and top parts. There were no significant differences in total sterol content among samples treated with MH-30 or Penar when compared with the untreated controls. Rates of Penar applied to the tobacco plants (100 mg once and 100 mg twice) had no effect on total sterol content.

10. Variation in sterols among flue-cured tobacco varieties. Genetic variation in sterol content was found in ten flue-cured tobacco varieties in research conducted at Florence, S.C., and Lexington, Ky. It should be possible to develop varieties with lower sterol content without affecting yield or alkaloid content.

11. Optimum conditions for bonding of plant phenols to insoluble polyvinylpyrrolidone. Insoluble polyvinylpyrrolidone (PVP) was studied at Lexington, Ky., because of its possible use as a specific precipitating agent for plant phenols in an analytical method for total phenols. Maximum bonding of the principal plant phenols of Nicotiana tabacum to insoluble PVP occurred at pH 3.5. Three successive additions of 0.5 g each of purified PVP quantitatively bound 100 microgram amounts of each phenol. The bonding was reversible. 8M urea, 5M guanidine hydrochloride and N-methyl-2-pyrrolidone released undergraded caffeic acid from the PVP insoluble complex. Analytical and spectral data indicated that the phenols were attached to the PVP principally by hydrogen bonds.

12. Biosynthesis and metabolism of rutin and certain sterols. Determinations of membrane permeability were made at Lexington, Ky., on root-tip cells of Beta vulgaris. Alcohols increased the permeability of the cell membranes without going through an induction period except for methanol which required a 10 - 15 hour induction period. The membrane effect of methanol could be inhibited with CaCl_2 , cholesterol, B-sitosterol and stigmasterol. Cholesterol was the most effective inhibitor, followed by B-sitosterol and stigmasterol; and at the same concentration, the sterols were more effective than CaCl_2 - the classic membrane stabilizer. Ergosterol increased the methanol-initiated betacyanin leakage. Results are explained in terms of a sterol-phospholipid interaction, based on stereochemistry and charge distribution.

13. Effects of agronomic factors on health-related compounds in Burley tobacco. Burley 21 tobacco was grown at Lexington, Ky., in different soil types, with varying amounts of nitrogen fertilization. One location also included the addition of growth regulators to the treatments. Growth of tobacco was slower in high N than in low N plots during much of the growing season. Differences due to varying nitrogen rates were greatest when the plants were small and decreased as they became larger. At the end of the season the plants on the high N plots were larger but showed symptoms of manganese toxicity.

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Tobacco

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TOBACCO INSECTS
Entomology Research Division, ARS

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years FY 1968		
	207	701	Total
Florida	1.9	.1	2.0
Maryland (Beltsville)	.3		0.3
North Carolina	4.0		4.0
South Carolina	.9	.1	1.0
St. Croix, V.I.	1.0		1.0
Total	8.1	.2	8.3

Intramural program is supplemented by extramural support representing 1.6 SMY's at State Agricultural Experiment Stations^{1/}.

1/ RPA 207 - 1.6

Problems and Objectives

Insecticides used for tobacco insect control may cause undesirable residues and off-flavors in cured tobacco. Residues may remain in or on the leaf during commercial processing and some have been found in the mainstream of smoke from commercial cigarettes. In addition, many of the insects previously controlled with chemicals have become resistant to all currently registered materials. Noninsecticidal methods for controlling tobacco pests are urgently needed.

The objectives of the research are to develop and utilize for the control of tobacco insects:

1. Selective, nonpersistent chemicals.
2. Insect resistant tobacco germ plasm.
3. New approaches such as sterility, attractants, and biotic agents.
4. Integrated control programs using methods such as chemicals, light traps, sterility, parasites, predators, and pathogens.

Progress - USDA and Cooperative Programs

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

A. Basic Biology, Physiology and Nutrition (1.2 SMY)

1. Tobacco Hornworm. At Oxford, N.C., successful laboratory mating of Apanteles congregatus, a braconid parasite of the tobacco hornworm, was obtained through control of ambient light and provision of adequate horizontal resting surfaces. Mating occurred primarily in the afternoon and oviposition in the morning. First and second instar hornworm larvae were preferred for oviposition. A complete life cycle was obtained for the first time in the laboratory.

In field-collected tobacco hornworm larvae at Oxford, 95% of the Tachinid parasite eggs were laid on the first 3 segments; however, in the laboratory on hornworms on artificial diet, 81% of the eggs were laid on segments VII to XI. Field-collected larvae averaged 24 eggs and lab-reared only 9.

At Oxford, artificial leaves used as oviposition sites for the tobacco hornworm gave consistently good results. Crude extracts of tomato or of tobacco leaves sprayed on artificial leaves stimulated oviposition, and in several trials artificial plants were more effective in inducing oviposition than the natural hosts. During oviposition experiments, the surrogates were as effective as the NC 95 tobacco variety, and were more effective than other varieties. Tobacco plants grown in greenhouses were seldom as effective as the artificial plants.

In experiments to determine factors influencing the selection of oviposition sites by tobacco hornworm females, a combination of aroma and moisture (leaves moistened with crude tobacco extract) was found to be the most effective oviposition stimulus.

Gravid female hornworms exposed to artificial oviposition sites under low light levels (0.1 ft-c) selected sites on the basis of visual stimulation, alone. Females visited untreated artificial leaves, sheathed in black or white felt to simulate hairy surfaces of natural host plants. A few eggs were deposited on untreated leaves, indicating that tarsal contact, alone, is sufficient stimulus for some moths. White leaves were preferred to black leaves, suggesting that reflectance may be important in the selection of oviposition sites. The use of both treated and untreated, camouflaged, artificial leaves in several experiments indicated that the female navigates to specific leaf sites primarily by vision, although a short-range olfactory stimulus is necessary for landing. The females also responded to shadows cast by the overlying leaves on an underlying white felt surface. Visiting females approached these shadows in the same manner as approaching host plant leaves. Several eggs were deposited around the edges of the shadows.

Of more than 50 dyes screened at Oxford for staining of the tobacco hornworm spermatophore in vivo, only Rhodamine B, pararosaniline (basic, acetate, and HCl forms), acid fuchsin, and methylene blue chloride were effective. No toxic effects resulted from 0.1% concentrations of these dyes. Although all of the first 3 dyes stained the spermatophore red, Rhodamine B was distinguishable by its fluorescence under ultraviolet light.

At Oxford, a new technique for mass rearing the tobacco hornworm on artificial diet showed promise of greatly increasing efficiency through the exclusion of contaminants, and avoiding the need for diet changes and handling of the larvae. In preliminary trials prepupal yields ranged from 70 to 90% of 200 larvae transferred compared with 50% or less with the previous method.

In studies at St. Croix, V.I., tobacco hornworm eggs were held at 60° F for 8 days without appreciably reducing their viability. When offered a choice, adult tobacco hornworm moths consumed twice as much of a 25% honey-water solution as a 25% dextrose-water solution, and seven times as much honey solution as beer.

An extensive search on St. Croix for wild host plants of the tobacco hornworm revealed it to be feeding on Solanum caribaeum and Solanum torvum.

A time interval trap operated on St. Croix showed that maximum activity of the tobacco hornworm occurred between 8:00 p.m. and 1:00 a.m.; 68.6% of the moths were caught between these hours.

In releases of 674 marked male moths over a 12-day period on St. Croix, blacklight traps baited with virgin females captured 151, or 22% of the

released moths. Moths dispersed widely from each release site. The releases demonstrated that use of the sterile male method would require no more than 3 release sites for the entire 84-mile² island and that baited traps were comparatively efficient in capturing male moths.

2. Cabbage Looper. At Quincy, Fla., increasing the wheat germ and substituting Torula for Brewer's yeast in Shorey's bean diet for the cabbage looper reduced the cost from \$1.29 per gallon to \$0.85, in addition to improving yield, size, and rate of development of the looper larvae. Further savings are anticipated from the substitution of Gelcarin for the standard agar. Facilities now permit rearing of 50,000 cabbage loopers weekly.

B. Insecticidal and Cultural Control (.6 SMY)

1. Wireworms. At Florence, S.C., new equipment was developed for applying insecticides to control wireworms in tobacco soil. A tractor equipped with one distributor spreads the insecticide granules on the soil and covers them to a depth of 3 to 4 inches by use of two sets of disk hillers and two large sweeps, operated behind the rear wheels of the tractor.

In field experiments, Union Carbide UC-21149, Bay-37289, Dyfonate, Mocap, carbofuran, and Dasanit gave consistently effective wireworm control when applied as granules.

2. Flea Beetles, Budworm, Hornworm. At Florence, the most effective insecticide for the control of foliage feeding tobacco insects was carbofuran. Applied to the soil in granules, it gave good control of tobacco flea beetles, budworms, and hornworms for several weeks. Granular disulfoton applied to the soil also gave effective control of the tobacco flea beetle and the green peach aphid.

Field plot experiments at Florence indicated good control of tobacco hornworm, tobacco flea beetle, and green peach aphid with dust or spray applications of a combination insecticide mixture leaving little residue on the treated plants. The insecticide mixture contained Mobam, rotenone, and parathion.

C. Biological Control (.8 SMY)

1. Tobacco Budworm and Cabbage Looper. At Quincy, Fla., parasitism of Heliothis eggs by a local species of Trichogramma (T. prediosum) showed 9% on tobacco and 37% on corn in a 7-acre cage. Weekly aerial applications of 2 quarts of Thuricide (Bacillus thuringiensis) per acre applied to cigar wrapper tobacco was as effective as the conventional insecticide treatment in controlling tobacco budworms and cabbage loopers.

D. Insect Sterility, Attractants, and Other New Approaches to Control (1.7 SMY)

1. Tobacco Hornworm and Budworm. At Oxford, N.C., 8,990 sterile male hornworms were released in an 80-mile² area encircled with a 5-mile-wide band of 3 blacklight traps per mile². After the first brood, male and female moth captures in the test area were 56 and 52% lower, respectively, than outside. Tomato hornworms, probably affected by sterile males, showed reductions of only 25% for males and 12% for females.

In tests of blacklight trap installation height ranging from ground level to 98 feet, catches of tomato and tobacco hornworms were high in the ground level trap. Indications were that such an installation effectively captures moths falling to the ground after they are attracted to traps located at higher levels.

Use of a mark-release-recapture method in a 19-mile² area of Johnston County, N.C., during the period June 29-September 1, 1965, provided estimates of approximately 23,630 male and 22,590 female tobacco hornworm moths in the area. During 1967, there was an estimated 28,546 pairs of moths in 98,470 acres of land or 9.35 per acre of tobacco from June 2 to September 1.

In Oxford tests, the tobacco hornworm moths marked with fluorescent paint applied as an aerosol were visible at night 100 ft from a blacklight lamp emitting in the range of 3200 - 3800 Å.

During 1962 and 1967 in two areas near Oxford, movement and survival of tobacco hornworm moths were compared with use of a mark-release-recapture method. Of 13,824 moths released, 260 (1.9 percent) were recaptured. The maximum distance of assumed natural flight was 6.8 miles; the mean distance traveled was 1.7 miles. The limited data available indicate that female moths disperse as widely as males. The dispersal pattern was about the same for laboratory reared moths as for wild moths, which were trapped, marked, released, and recaptured. In one night moths became well distributed over the area out to 3 miles from the release site.

At Oxford, blacklight traps spaced at 1-mile intervals in a 16-mile² area and baited with two virgin female hornworm moths caught four times as many males as unbaited blacklight traps. The ratio of male to female moths was greater in low population areas.

At Florence, S.C., extracts of the abdominal tips from virgin female tobacco hornworms were attractive to males in field studies.

On St. Croix, after 2 years of trapping with 3 blacklight traps per mile² over the entire island, tobacco hornworm populations were approximately 30% as high as recorded during the year prior to the trapping program.

On St. Croix, 2 virgin female hornworms placed at a blacklight trap increased the male tobacco hornworm collection by 7 times and 4 virgin females increased the collection by 10 times. Blacklight traps baited with virgin females and placed 10 feet high collected 1.7 times more male moths

than when placed 5 feet high, and 2.3 times more than those placed 1 foot high. Crude extracts of the virgin females were ineffective in increasing male captures. A comparison of a North Carolina and St. Croix strain revealed that, although virgin females of both strains effectively increased captures of male hornworm moths, the St. Croix strain was the most effective.

2. Heliothis sp. At Quincy, Fla., Heliothis zea and H. virescens moths were released in a 7-acre cage containing 5 evenly-spaced standard blacklight traps equipped with chemosterilant feeders (tepa-sucrose solution). After 3 days, the percent reduction in egg hatch compared with a 1-acre control cage was 70% for H. zea eggs collected from corn and 9% from H. virescens eggs collected from tobacco. Eggs from moths of both species collected from the 7-acre cage 3 days after release showed a reduction in hatch of 39% for H. virescens and 91% for H. zea.

In an area of approximately 1/2-mile², 26 blacklight traps each baited with 10 virgin female H. virescens moths showed a substantial increase in male catch over unbaited blacklight traps.

3. Cabbage Looper. At Quincy, Fla., populations of female cabbage looper moths were higher inside a 1-mile² area containing 60 blacklight traps, each baited with .1 gram of synthetic sex pheromone, than in outside areas with installations of 3 unbaited blacklight traps per mile². Spermatophore counts showed that 30% of the females collected from traps inside the 1-mile² area had mated while 52% of the females taken from traps outside were mated. Total mating within the 1-mile² area was 26% less than in outside areas. However, there was no difference in egg and larval counts or in percent egg hatch within and outside the area.

4. Miscellaneous Tobacco Insects. At Quincy, Fla., during the 1965, 1966, and 1967 growing seasons, integrated insect control programs were conducted using disulfoton, light traps, the pathogen Bacillus thuringiensis, and nonpersistent insecticide treatments as needed. Compared to conventional programs, the integrated programs reduced the total number of seasonal insecticide applications on cigar wrapper tobacco by 80, 76, and 43 percent, respectively. There was no significant difference in plant damage to tobacco between the integrated programs and the conventional insecticide program. In 1967, local cooperators grew 71 acres of tobacco under the integrated program and showed a saving of approximately \$26.00 per acre compared with the regular insecticide program.

Blacklight traps were spaced around the perimeter of a cigar wrapper tobacco field in conjunction with weekly aerial applications of the pathogen Bacillus thuringiensis beginning 2 weeks after transplanting. The mean number of plants damaged by tobacco budworms and cabbage loopers was .88 plants per 100 plants examined as compared with 5.5 plants damaged in fields under conventional insecticide programs where insecticide was applied once or twice per week. When the treatment in conjunction with the blacklight traps consisted of weekly applications of 1% parathion and

10% DDT, the mean number of plants damaged was .13 compared with 6.0 under the conventional insecticide program.

E. Evaluation of Equipment for Insect Detection and Control (.1 SMY)

At Florence, S.C., soil samples indicated higher larval populations of the southern potato wireworm near blacklight traps than in areas not associated with traps. Apparently a significant portion of the adults attracted are not trapped.

F. Varietal Evaluation for Insect Control (.2 SMY)

At Oxford, N.C., approximately 80 species of Nicotiana and 160 varieties of N. tabacum were tested to determine potential resistance to tobacco hornworms and budworms.

RPA 701 - INSURE FOOD PRODUCTS FREE OF TOXIC RESIDUES FROM AGRICULTURAL SOURCES

A. Insecticide Residue Determinations (.1 SMY)

At Florence, S.C., in cooperation with industry, no off-taste and flavor was shown in cured tobacco samples after soil treatments for wireworms with diazinon wettable powder, and granular formulations of diazinon, Bay-37289, Dasanit, Union Carbide UC-21149, Mocap, or carbofuran.

Publications - USDA and Cooperative Programs

RPA 207 - CONTROL OF INSECT PESTS OF FIELD CROPS

Basic Biology, Physiology and Nutrition

Carlson, S. D., Smith, J. S., and Stanley, J. M. 1968. Moth visual potentials in response to middle UV-radiation. *Experientia* 24: 289-91.

Carlson, S. D., Steeves, H. R., and VandeBerg, J. S. 1967. Vitamin A deficiency: Effect on retinal structure of the moth Manduca sexta. *Sci.* 158: 266-70.

McFadden, M. W. 1968. Observations on feeding and movement of tobacco hornworm larvae. *J. Econ. Entomol.* 61: 352-6.

Stewart, P. A., Lam, J. J. Jr., and Hoffman, J. D. 1967. Activity of tobacco hornworm and corn earworm moths as determined by traps equipped with blacklight lamps. *J. Econ. Entomol.* 60: 1520-2.

Yamamoto, R. T. 1968. Mass rearing of the tobacco hornworm. I. Egg production. *J. Econ. Entomol.* 61: 170-4.

Insect Sterility, Attractants, and Other New Approaches to Insect Control

Gentry, C. R., Lawson, F. R., Knott, C. M., Stanley, J. M., and Lam, J. J. Jr. 1967. Control of hornworms by trapping with blacklight and stalk cutting in North Carolina. *J. Econ. Entomol.* 60: 1437-42.

- Hayes, Sidney B. 1968. Adult hornworm populations and degree of infestation on tobacco in relation to community-wide grower use of blacklight traps. *J. Econ. Entomol.* 61: 613-7.
- Hoffman, J. D., Lawson, F. R., and Yamamoto, R. T. 1966. Tobacco hornworms, p. 479-86. In *Insect Colonization and Mass Production*. Acad. Press Inc., New York.
- Stewart, P. A. and Lam, Jesse J. 1968. Marking tobacco hornworm moths for night recovery with a blacklight lamp. *J. Econ. Entomol.* 61: 864-5.
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Varietal Evaluation for Insect Control

- Stewart, P. A. 1968. Testing tobacco varieties for resistance to hornworms. *Tobacco Sci.* 12: 5-6.

ENGINEERING ASPECTS OF TOBACCO PRODUCTION
Agricultural Engineering Research Division, ARSUSDA and Cooperative Program

Research Problem Area	:	Location of Intramural Work	:	Scientist-Man- Years FY 1968	
207 - Control of Insect Pests:	:	Florida (Quincy)	:	0.0	
	:	North Carolina (Oxford)	:	1.0	
	:	Virginia (Blacksburg)	:	1.0	
	:		:		
308 - Mechanization:	:	Kentucky		2.0	
			TOTAL	:	4.0

Problems and Objectives

The economic loss of field crops from insect damage was estimated at \$19 billion in 1964 despite the effects of insect-control programs. To reduce this loss, in the face of increasing difficulty in controlling insects as they become resistant to insecticides and increasing concern about chemical residues in the environment, it is essential that existing chemical materials be applied most effectively and that nonchemical control methods be thoroughly evaluated.

Engineering objectives of the research program include:

1. Development and evaluation of improved methods for applying insecticides.
2. Devising electrical methods and equipment for insect control.
3. Devising mechanical methods and equipment for direct insect control or for rendering host-plant material unfit for insects.
4. Evaluating uses of control principles in combination.

In considering the three major items of capital outlay for the production of food and fiber crops--land, buildings, and machinery--it is generally conceded that the machinery costs exceed the others by a wide margin. Improvements and changes in present day farm machinery are continually taking place in order to adapt to new varieties, improved production practices, a need for higher efficiency, and changing handling and storage practices. The cost of owning and operating field equipment for most crops is the major expense of production, in some cases, amounting to nearly half the total returns to the producer from the sale of the crop. Harvesting equipment is the most sophisticated and highest cost of all agricultural machinery, requiring expert attention by the operator during its period of use. The four billion man-hours of labor and the six billion dollar machine costs used annually in operating American farms offers ample potential for fruitful results.

The major objectives of research on the mechanization of field crops include:

1. The development of machinery and improved machine components to plant, cultivate, harvest, and handle specific crops in order to minimize power requirements, reduce the number of field operations and provide optimum seedbed, growing, and harvesting conditions.
2. The development of precision devices and automated systems that reduce labor requirements.

3. The determination of the rheological properties of crops that relate to the harvesting, handling, and processing in order to design and adopt labor saving devices.
4. The modification of varieties and cultural practices to provide plants more easily adopted to mechanized operations and labor saving devices.
5. The development of more efficient automated systems that encompass all field and post harvest operations that handle, cure, dry, clean, gin, or otherwise prepare the crop for market.

Progress - USDA and Cooperative Programs

1. Insect control in shade-grown tobacco. An integrated insect-control program was tested that included insect light traps spaced at 150-foot intervals around the outside of canvas-covered tobacco fields, a systemic insecticide applied to the soil prior to transplanting, and nonpersistent insecticide treatment of the growing crop as needed. Damaged-plant data from eight other fields of tobacco that received one to two applications of insecticide weekly, considered to be a conventional program, indicated that the damage from tobacco budworms and cabbage loopers was 2.8 times greater than the plant damage in seven fields under the integrated control program. The integrated program reduced the amount of insecticide needed by a significant amount. Synthesized cabbage looper sex pheromone was incorporated with each of the 1,100 traps used in the program over the 400-square-mile shade-grown tobacco area. Cold weather reduced the insect activity after the test was started in the fall, but the results of the test were encouraging.

This work is cooperative with the ENT Division and the Florida Station, but at present AE Division has no professional participant.

2. Insect response studies. Related work is conducted with the Research Division of Virginia Polytechnic Institute, partially under Cooperative Agreement.

In laboratory studies on the attraction of insects by radiant energy, electro-physiological tests established the spectral sensitivity curve for the tobacco hornworm moth. Response of the flight-muscle nerve is probably an indication of the moth's flight deviation in response to energy. Response appeared greater to ultraviolet than to visible energy. Similar examinations of signals in optic nerves were made for responses to radiant energy. Limited electroretinographic studies were conducted on tobacco budworm moths, yielding a sensitivity curve similar to that of the hornworm moth.

In field studies, a combination of green and blacklight (BL) fluorescent lamps was less effective in attracting nocturnal tobacco insects than BL

lamps alone. In experimental traps with three different-sized funnels nested together coaxially, it was found that the largest portion of the insects was collected in the central funnel, which was 18 inches in diameter. When unidirectional fan and gravity traps were operated under competitive conditions, gravity traps were more effective in trapping hornworm moths. In experiments comparing new and old lamps, no differences in catches were detected which could be attributed to lamp age. Neither were any differences detected between responses to BL lamps with two different types of phosphors. Major hornworm activity, as determined by light trap catches, was between 9 p.m. and 5 a.m., with the peak between 1 a.m. and 3 a.m.

Electrophysiological investigations with tobacco hornworm moths yielded no detectable evidence of response to sonic energy stimulations. Electrodes were placed in the cervical connective, antennal nerve, and Johnston's organ. Sonic treatments of 80-dB intensity ranges in frequency from 50 Hz to 100 KHz, pulsed at a rate of 10 pulses per second; and square-wave pulses, or "clicks," of 1-millisecond duration ranging from 1 to 100 pulses per second. Nerve responses were related more to elongation of an antenna than to deflection. Flight sounds of houseflies have been recorded and used as a stimulus, but no detectable response was obtained with flies.

3. Insect control--large-area trails. A large-scale field experiment on hornworm control is being conducted in cooperation with the North Carolina Station and the ENT Division.

In 1967, traps in the circular band of area between radii 5 and 10 miles from the area center were used to isolate the central 80-square-mile area in which sterile male tobacco hornworm moths were released to compete with native males. Although only 8,990 sterile males were released during the summer, tobacco hornworm moth populations, after the first brood, were lower inside than outside the release area by 56 and 52 percent, respectively, for males and females. Tomato hornworm moth populations were also lower inside than outside the release area by 25 and 12 percent, respectively, for males and females, indicating some residual effect from light traps during previous years but a population buildup when traps were removed. No major differences were measured for moth populations of tobacco budworm or corn earworm inside and outside the area in 1967.

In tests with caged moths, the threshold of response to a 15-watt blacklight source was between 91.4 and 121.9 meters from the energy source for tobacco hornworm moths and between 61.0 and 91.4 meters for the corn earworm moth.

Data collected in similar experiments in cooperation with ENT Division over a 22-month period on St. Croix indicate that using insect light traps with one 15-watt blacklight fluorescent lamp will reduce the population of Maduca sexta when installed at a density of approximately three traps per square mile. Additional data will help to determine whether these traps will control the population of this moth. Trap catches are greater during the dark period of the moon's cycle. Trap location appears to influence the catch. Weather factors also influence the catch. Equipment is being

installed to monitor wind speed and direction, temperature, relative humidity, rainfall, and lunar illumination. These will be correlated with moth catches to determine their influence on moth activity. Various numbers and placement of lamps will be evaluated in improving trap efficiency.

4. Burley tobacco harvesting equipment and methods. Hydraulic control and power circuits have been designed for a stalk-cut harvester having the function to fasten base of cut stalks to continuous twine. A procedure has been developed for handling stalk-cut tobacco using portable curing frames, forklift agricultural tractors, and air-cure barns reduces housing labor from 40 to 20 man-hours per acre. Marketing of a steel frame is proposed by a project cooperator. Equipment has been designed for artificially casing cured tobacco on the frames, allowing scheduling of market preparation, independent of weather. Value of experimentally cured tobacco has been equivalent to conventionally handled and cured tobacco. In 1967 a two-acre demonstration farm was operated by a cooperator.

5. Burley tobacco curing equipment and techniques. Programmed curing of primed Burley tobacco by a standard drying curve which was selected on the basis of previous research, was satisfactory. Curing temperatures of 80° F. and 90° F. were compared where the drying curves were the same. Tobacco cured at 90° F. obtained a higher grade. Respiration heat generated by Burley tobacco during the cure was measured at air temperatures of 80°, 90°, 110° F. The respiration rate reached a maximum of 13 to 18 B.t.u./hr./lb. dry matter after 25 to 35 hours of curing time. The maximum rate increased with temperature. The total quantity of respiration heat liberated in two weeks was the same for all temperatures. The dry matter equivalent of total heat liberated was about 20 percent of the dry matter. This heat was sufficient to vaporize about 40 percent of water which was removed from the tobacco. The moisture gain with time for temperatures of 40° F. to 70° F. and humidities of 75 percent to 95 percent r.h. were determined for cured Burley plants, leaves, and stalks. For a constant relative humidity, the time required to reach a constant moisture content decreased as temperature increased. For a constant temperature, the time required to reach a constant moisture content increased as humidity increased.

Publications - USDA and Cooperative Program

Mechanical Insect Control

GENTRY, C. R., LAWSON, F. R., KNOTT, C. M., STANLEY, J. M., and LAM, JR., J.J. Control of hornworms by trapping with blacklight and stalk cutting in North Carolina. *J. Econ. Entomol.* 60(5):1437-1442. Oct. 1967.

STEWART, P. A., GENTRY, C. R., KNOTT, C. M., and LAM, JR., J. J. Seasonal trends in catches of moths of the tobacco hornworm, tomato hornworm, and corn earworm in traps equipped with blacklight lamps in North Carolina. *J. Econ. Entomol.* 61(1):43-46. Feb. 1968.

STEWART, P. A., LAM, JR. J. J., and HOFFMAN, J. D. Activity of tobacco hornworm and corn earworm moths as determined by traps equipped with black-light lamps. J. Econ. Entomol. 60(6):1520-1522. Dec. 1967.

Harvesting and Curing Mechanization

YODER, E. E. New labor saving device. The Burley Tobacco Farmer. Summer Quarter 1967.

BUNN, J. M., HENSON, W. H., JR., and SMITH, E. M. Effects of curing techniques on the hygroscopic properties of primed Burley tobacco. Tobacco Science, Vol. XI, pp. 124-129. Sept. 1967.

II. CONSUMER AND INDUSTRIAL USE RESEARCH

TOBACCO UTILIZATION

Eastern Utilization Research and Development Division, ARS

USDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968			
	Research Problem Area		Total	:
	709	407		
Pennsylvania (Wyndmoor)	:	:	:	:
A. Chemical Composition, Physical Properties and Structure	:	8.9	0.2	9.1
B. Chemical and Physical Investigations to Improve Products	:	6.1	0	6.1
C. Microbiology and Fermentation	:	4.2	0	4.2
D. Technology - Process and Product Development	:	1.0	0	1.0
Total	:	20.2	0.2	20.4

Intramural program is supplemented by extramural support representing (a) 17.1 SMY's at universities and at state agricultural experiment stations^{1/}, and (b) 3.0 SMY's at other U. S. institutions^{1/}.

^{1/} RPA 709 - Chemical Composition, Physical Properties and Structure, 4.2; Chemical and Physical Investigations to Improve Products, 10.0; and Technology - Process and Product Development, 5.9.

Problems and Objectives

Tobacco is grown on about a million acres, and in seven states typically provides more farm cash receipts than any other field crop. The most serious problem affecting the tobacco industry is the concern regarding effect on health.

Major shortcomings in developing a safer cigarette are the inadequacies of biological testing procedures and the failure to know which substances in tobacco and smoke should be removed. There is no practical procedure for determining which substances in tobacco and tobacco smoke are injurious to public health. Based on limited knowledge, some currently marketed cigarettes with charcoal and other filters apparently show selective reductions in certain properties in laboratory animals but cannot be considered a final solution. In addition, such cigarettes are deficient in flavor and aroma and meet with some consumer resistance. This problem requires a concerted effort to learn specifically what must be removed from cigarette smoke and how this can be done effectively without loss of desirable organoleptic properties.

Objectives of research are to:

1. Develop methods for determining which substances in tobacco and tobacco smoke are undesirable from standpoint of public safety, and
2. Develop technology for elimination of substances determined to be undesirable.

Progress - USDA and Cooperative Program

RPA 709 - REDUCTION IN HEALTH HAZARDS INVOLVED IN USE OF NONFOOD FARM PRODUCTS

A. Chemical Composition, Physical Properties and Structure

1. Composition of Tobacco Smoke. A quinone, 2,3,6-trimethyl-1,4-naphtho-quinone was isolated for the first time from cigarette smoke. This compound was obtained in very small quantity from the nitromethane-soluble neutral fraction of smoke condensate and was identified by spectral characteristics and by similarity to the synthesized compound. 9-Fluorenone, recently reported in smoke from dark tobaccos by French workers, was identified also as a component of this neutral fraction. A fractionation procedure was developed which enables considerably greater concentration of the polynuclear hydrocarbons than was possible heretofore. Biological tests can now be initiated on the subfractions of the neutral fraction to determine the role of polynuclear hydrocarbons, and other neutral compounds, in carcinogenic and cocarcinogenic activity.

The isolation of four aromatic amines from cigarette smoke condensate, previously reported, prompted a search for the highly carcinogenic beta-naphthylamine. This compound was not found. On the basis of model recovery experiments it was concluded that beta-naphthylamine, if present at all, does not occur in amounts greater than 0.04 μ g per cigarette.

A basic pigment isolated from cigarette smoke condensate was shown to differ from the previously isolated acidic pigment in content of quinic acid and hydrolysis products (amino acids and nitrogenous bases).

At the University of Kentucky Research Foundation, Lexington, Kentucky, under contract research, cigarette smoke condensate was divided into a volatile fraction and a nonvolatile fraction by distillation at reduced pressure. These fractions were separated into a water-soluble and a water-insoluble fraction. The basic fraction was separated from the water-insoluble fraction and subjected to gas chromatographic analysis. About 50 compounds eluted prior to nicotine and at least 62 compounds after nicotine. Pyrrolidine, pyridine, pyrrole, picolines, lutidines, 3-vinylpyridine, 3-ethylpyridine, 2,4,6-collidine, 3-acetylpyridine, and quinoline were tentatively identified.

In research under another contract with University of Kentucky Research Foundation, a method was developed for determining the amounts of anthracene and pyrene in cigarette smoke. Smoke condensate was separated on a silic acid column. The first fraction (band) was further chromatographed on Sephadex LH-20, and the amounts of anthracene and pyrene were determined by gas chromatography. In the smoke of one hundred, 85 mm., unfiltered cigarettes were found 20.3 μ g of anthracene and 21.5 μ g of pyrene. Eight other previously reported polynuclear hydrocarbons were also identified by this method.

Also, at Lexington, Kentucky, potassium antimonate and potassium carbonate plus potassium hydroxide columns were employed for the first time in gas-solid chromatography of polynuclear aromatic hydrocarbons. The column containing $K_2Sb_4O_7$ gave good separation of an authentic mixture of phenanthrene, anthracene, 1-methylphenanthrene, 2-methylphenanthrene, and pyrene. Good separations of anthracene and phenanthrene were achieved on both the potassium antimonate and potassium carbonate plus potassium hydroxide columns. The amounts of phenanthrene and anthracene present in a cigarette smoke condensate fraction were determined gas-chromatographically using the antimonate column. In the smoke of one hundred 85 mm. commercial cigarettes without filter tip were found 39.6 μ g of phenanthrene and 13.0 μ g of anthracene.

Seven different nitrosamines were synthesized and their spectroscopic and gas chromatographic patterns were determined. The stability and the distribution coefficient of dimethylnitrosamine under conditions normally encountered in the fractionation of cigarette smoke condensate were investigated. Distribution coefficients of three other nitrosamines between ether and water were also determined. Supplementary data indicated that the occurrence of nitrosamines in the low boiling fraction of cigarette smoke condensate, or a steam volatile fraction of smoke condensate, was either nil or limited to

trace quantities.

2. Composition of Tobacco Leaf. The brown tobacco pigments were isolated from a large-scale extract of Turkish tobacco. Potassium hydroxide fusion of the nondialyzable fraction of the pigment yielded a variety of volatile bases and a silicone. Compositional studies on the leaf pigments of the four principal cigarette tobacco types have shown the pigments to be similar in physical and chemical characteristics. Studies were also initiated on the phenolic compounds of tobacco leaf. Extracts of flue-cured tobacco have been prepared and submitted for bioassay tests at the Roswell Park Memorial Institute, Buffalo, New York. Solvents of increasing polarity (petroleum ether, chloroform, acetone, ethanol, methanol, water) were used in the consecutive extractions.

In contract research at the Research Triangle Institute, Durham, North Carolina, the glyceryl ester fraction of the hexane extract was separated from the sterol ester fraction by gel permeation. At least 1% of the hexane extract consists of the glyceride fraction whereas the sterol esters make up a minimum of 1.5% of this extract. Acids in the glyceride fraction were tentatively identified as myristic, palmitic, stearic, oleic, linoleic, and linolenic by gas chromatographic analysis of the methyl esters. These studies indicate that the neutral resins of leaf contain many components capable of serving as potent precursors of polynuclear aromatic hydrocarbons (PAH) of smoke. On pyrolysis, the isolated sterols and cyclic triterpenes may easily fragment, dehydrogenate and react with other radicals or groups to give PAH and thus contribute disproportionately to the overall PAH levels of smoke. The presence of free α - and β -amyrin and free or esterified cholesterol in leaf has not been reported previously.

B. Chemical and Physical Investigations to Improve Products

1. Cigarette Modifiers. Cooperative research at the University of Kentucky, Lexington, has shown that the addition of various chemical additives to tobacco results in alterations of the levels of certain smoke constituents. Additives include chemicals such as flame-retardants, free-radical scavengers, oxidants and temperature depressants. Some changes in the level of possible health-related smoke constituents have been detected. Decreases of 27% for total particulate matter (tar); 42% for nicotine; 34% for phenol and 71% for benzo(a)pyrene were obtained. For the majority of modifiers evaluated thus far, the most consistent alteration is the reduction in total particulate matter.

Cigarettes containing 8.3% NaNO_3 were prepared and smoked on a newly-constructed smoking apparatus. The smoke (vapor phase) generated from these cigarettes was collected in an appropriate collection flask and analyzed by gas chromatographic and/or colorimetric methods. The addition of NaNO_3 to cigarettes was shown to produce relatively large quantities of undesirable vapor phase constituents such as nitrogen oxides, acetaldehyde, acrolein and acetonitrile. All of these increased by at least a factor of two in smoke from treated cigarettes (vs. control). The smoke of treated cigarettes in

addition contained 160 ppm of N_2O , whereas the quantity present in smoke from control cigarettes was too low to be detected. Addition of $NaNO_3$ also had the effect of increasing levels of olefinic hydrocarbons in smoke, while levels of CO, HCN, HCHO and paraffins remained unchanged. Other effects of $NaNO_3$ addition were (1) reduction of H_2S levels in smoke from 4.5 to 0.3 $\mu g/$ puff and (2) lowering of burn temperature from 850°C. to 788°C.

2. Pyrolytic Products. Pyrolytic studies at EU indicated some of the non-volatile acids in tobacco leaf may serve as phenol precursors. Sodium lactate gave relatively high yields of phenols. Thus far, many types of organic materials gave rise to phenols (phenol, o-cresol, m- and/or p-cresol, and others) under appropriate pyrolytic conditions. Tobacco leaf pigment yielded a variety of N-heterocycles but no nicotine. It appears that in a burning cigarette, other processes (e.g. distillation) in addition to pyrolysis influence smoke composition. Polynuclear aromatic hydrocarbons (PAH) can be formed from any compound containing C and H above temperatures of 600°C. Pyrolyses in air formed more complex PAH mixtures than those conducted in nitrogen. Cinnamic acid and similar compounds produced t-stilbene as a major component and at least 30 other compounds which were mainly PAH. A method was developed for analyzing many acids in pyrolysates as well as in tobacco products.

In contract research at the University of Kentucky Research Foundation, pyrolysis of lysine monohydrochloride and phenylalanine at 850°C. in nitrogen yielded small amounts of phenol. Lysine monochloride, leucine, phenylalanine, and tryptophan formed, upon pyrolysis, naphthalene, acenaphthalene, fluorene, fluoranthene, pyrene, benzofluorenes, chrysene, benzo(a)pyrene, anthracene, phenanthrene, picene, and at least six other polynuclear hydrocarbons. Low molecular weight compounds such as methane, ethylene, acetylene, and carbon monoxide were also formed. Composition of the pyrolytic products was dependent upon the temperature of pyrolysis and the starting material. Yields of polynuclear hydrocarbons varied directly with pyrolysis temperature whereas heterocyclic bases were formed in highest yield at the lower temperatures.

Glutamic acid, proline, serine, alanine, 2-aminobutyric acid, valine, and phenylethylamine were pyrolyzed at 650° and 850°C. Yields of condensate varied inversely with the temperature of pyrolysis, but there was no consistent trend between pyrolysate yield and the weight of constituent fractions (phenols, acids, bases). Yields of these constituent fractions and of the individual gaseous products were apparently more dependent upon amino acid structure. HCN was formed in highest yield from amino acids having branched chains or numerous functional groups.

3. Biological Assay Methods. In the development of new and improved biological assay methods under contract research at the University of Kentucky Research Foundation, studies on tracheal rings grown in tissue culture have demonstrated cellular responses to acetone-soluble smoke condensate and a partial recovery upon cessation of toxicant administration. The water-soluble smoke extract has shown ciliostatic properties which are concentration-related. It may be possible to develop a whole family of ciliostatic

dose-response curves for different fractions of the water-soluble smoke extract for preliminary screening of tobacco products.

Epithelial cell turnover rate is being investigated with two strains of mice. One strain exhibits a low incidence and the other a high incidence of spontaneous lung tumors. In order to avoid the possibility that the data may be biased due to the method employed to count the dividing cells, two different methods are being used. One of these employs colchicine to arrest the cells in metaphase; the other utilizes tritiated thymidine to label those cells in the process of dividing. In order to obtain meaningful data 300 animals are being studied and a minimum of 15,000 cells are being counted per tissue. Preliminary findings indicate that both the colchicine and tritiated thymidine methods yield similar data. Each show that the turnover rate of bronchial epithelial cells in mice is surprisingly low, ranging from 222 to 690 days. The turnover rates for mice with a high incidence of spontaneous lung tumors is, however, lower than that for mice exhibiting a low incidence of spontaneous lung tumors. There appears to be no difference in the turnover rates on the basis of the sex of the animals.

Tumor development in the cheek pouch of hamsters was achieved with low doses of 9,10-dimethylbenzanthracene which had been protected against UV radiation.

Cell-free homogenates of 3-methylcholanthrene-induced sarcomas enhanced tumor formation when applied with a chemical carcinogen. These results together with previous findings lends support to the theory that some host factor, perhaps a virus, is involved.

Ingestion of phenanthrene prior to feeding labeled benzo(a)pyrene to rats resulted in lower levels of radioactivity in the liver than in animals receiving no phenanthrene, indicating competition of these compounds in certain metabolic pathways. No change was found when phenol was administered one hour prior to the labeled benzo(a)pyrene.

Absorption and rate of transport in rat intestine of three strong carcinogens (benzo(a)pyrene, 3-methylcholanthrene and 7,12-dimethylbenzanthracene) was greater than that for noncarcinogenic or weakly carcinogenic compounds. For benzo(a)pyrene the rate of transport into and across the intestinal mucosa increased exponentially with increase in concentration of the hydrocarbon in the medium. As the transport of benzo(a)pyrene was found to take place under anaerobic as well as aerobic conditions, the transport mechanism does not require energy in the same way as the intestinal transport of polar molecules (e.g. amino acids and sugars).

Components of an electron transport system in Bacteriodes melaninogenicus are flavoproteins, cytochrome C, a carbon monoxide binding pigment, and possibly cytochrome B. Exposure of the system to the carcinogen 2-n-nonyl-4-hydroxy-quinoline-N-oxide inhibited electron transport between the flavoprotein and cytochrome C and inhibited growth. Exposure to carbon monoxide, a component of tobacco smoke, also inhibited electron transport and growth.

Mouse strains prone to respiratory infections have a higher incidence of accelerated spontaneous tumors than infection-resistant strains. It therefore is important that the animal species used for assessing carcinogenicity via intranasal exposure be as free as possible from respiratory infection. The use of hamsters is being investigated since these animals are naturally resistant to infection and spontaneous lung tumors in these animals are extremely rare. Experiments are under way to establish techniques for intranasal inoculation which will assure particulate distribution to the terminal air passages.

Experiments to determine the optimum dose of an aqueous extract of cigarette smoke condensate (AECSC) which would be tolerated by rats and compatible with near optimum weight gains have been accomplished. The data obtained clearly show that for all groups treated with doses of AECSC higher than 0.5 mg/ml, dehydration and loss of body weight occurs. In one group, given 5 mg/ml smoke condensate, a significant degree of leucopenia occurred. Whether this was a consequence of decreased protein intake or a direct effect of the smoke extract could not be determined from the data. In this group all tissues of the reticuloendothelial system (liver, spleen, and thymus) were lighter than the controls on the basis of equal body weights. However, the kidneys, heart, lungs adrenals, and brain were heavier. Of the several concentrations of AECSC investigated, near optimal weight gains were achieved only at the 0.25 mg/ml dose level. This dose or one slightly lower will therefore be used in all future experiments.

The first phase of the contract bioassay of smoke fractions by Health Research Inc., Buffalo, New York, has been completed except for microscopic examination of tissues of the animals, still under way. The major carcinogenic substances of smoke condensate were found to occur in three of the 12 fractions tested: the weakly acidic (phenolic) fraction and two neutral subfractions (cyclohexane-soluble and nitromethane-soluble).

C. Microbiology and Fermentation

Phosphate esters of carbohydrates and nucleotides have shown promise as indicators of metabolic activity differences between fermented and cured tobaccos. A column chromatographic procedure was used in resolving various nucleoside mono-, di-, and tri-phosphates. Further separation and purification was achieved by a linear gradient procedure. This methodology has been applied in preliminary experiments to the analysis of tobacco extracts. Peroxidase activity in a 1967 Pennsylvania filler cigar tobacco sample decreased 40-60% during 14 weeks of curing. Cured filler tobacco samples dating back to the 1962 crop still had measurable, but slight, peroxidase activity. Techniques have been developed for the growth of Aspergillus niger on cigarette tobaccos. Under laboratory conditions tobacco infected with A. niger did not produce aflatoxin. Previously reported metabolic products in mold-infected tobacco were identical with nontoxic metabolites found earlier by other investigators.

D. Technology - Process and Product Development

1. Modification of Cigarette Burn Temperature. About 90 different additives were evaluated as burn temperature modifiers in contract work at the Houdry Laboratories, Air Products and Chemicals, Inc. Basic magnesium carbonate, nickel oxalate, aluminum sulfate octadecahydrate, and halides or carbonates of sodium, potassium, and silver depressed coal temperatures. Free radical initiators, such as benzothiazyl disulfide and benzyl disulfide, increased coal temperatures. In general, relatively large amounts of derivatives (20-50%) were required to alter burn temperatures markedly. It appears that additives effect changes in tobacco combustion products without significant modification of the coal temperature.

2. Production of Smoke Condensates and Experimental Cigarettes. Smoke condensate is prepared and fractionated at EU in kilogram quantities for bioassay research under contract with Health Research, Inc. Currently the condensate is separated into twelve fractions for use in animal tests.

At Lexington, Kentucky, production of smoke concentrate from machine-smoked cigarettes is now sufficient to meet the program requirements for whole tar and fractions thereof at that location. The condensates may be modified, as for example by the removal of nicotine, to meet the requirements of a particular end use.

In the bioassay service for the investigations at the University of Kentucky, experiments to assess the biological activity of cigarette smoke condensate from high- and low-nitrate burley tobaccos are in progress. To date, no tumors have appeared on the backs of mice painted with either non-denicotinized or denicotinized condensate. This includes 11 mice which have been painted with non-denicotinized, high-nitrogen burley condensate for 13 months. Of 100 mice (controls) painted with 0.005% benzo(a)pyrene for 13 months, 12 mice out of 37 survivors have developed tumors.

RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

A. Chemical Composition, Physical Properties and Structure

1. Cigar Smoke. The mainstream smoke condensate from one thousand cigars has been fractionated under mild conditions. Indications of dienes, trienes, and possibly tetraenes in a hydrocarbon fraction have been obtained. Although many of these compounds are present in small quantities they may have significant organoleptic properties.

2. Cigar Residual Aroma. Major volatile chemical constituents of cigar butts were identified as acetonitrile, propionitrile, acetone, butanone, dipentene, 3-acetylpyridine, pyrrole, nicotine, and water. Twelve other organic compounds, which were present in lower concentrations, could also contribute to the residual aroma of the four cigar filler types under investigation. Colombian tobacco had the highest concentration of isoprene in the mainstream

smoke. Puerto Rican was highest in acetaldehyde.

- B. Chemical and Physical Investigations to Improve Products (no current research)
- C. Microbiology and Fermentation (no current research)
- D. Technology - Process and Product Development (no current research)

Publications and Patents - USDA and Cooperative Programs

RPA 709 - REDUCTION IN HEALTH HAZARDS INVOLVED IN USE OF NONFOOD FARM PRODUCTS

Chemical Composition, Physical Properties and Structure

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Stedman, R. L., Miller, R. L., Lakritz, L. and Chamberlain, W. J. 1968. Method for concentrating polynuclear aromatic hydrocarbons in cigarette smoke condensate. *Chem. & Ind.* 394-395.

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Chemical and Physical Investigations to Improve Products

Benner, J. F., Burton, H. R., and Burdick, D. 1968. Composition of cigarette smoke from high- and low-nitrate burley tobacco. *Tob. Sci.*, 12, 35-38.

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Microbiology and Fermentation

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RPA 407 - NEW AND IMPROVED FEED, TEXTILE AND INDUSTRIAL PRODUCTS FROM FIELD CROPS

Chemical Composition, Physical Properties and Structure

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III. MARKETING AND ECONOMIC RESEARCH

TOBACCO MARKET QUALITY
Market Quality Research Division, ARSUSDA and Cooperative Program

Location of Intramural Work	Scientist Man-Years F.Y. 1968				Total	
	Research Problem Area			:		
	408	501	702			
North Carolina	0.5	0.5	0.5	:	1.5	
Virginia (Insects)	1.0	:	:	:	1.0	
Total	1.5	0.5	0.5	:	2.5	

Intramural program is supplemented by extramural support representing (a) 5.8 SMYs at State Agricultural Experiment Stations 1/ and (b) P.L. 480 funds in one country representing 5,065 U.S. dollars equivalent.

1/ RPA 408 0.5; RPA 501 0.3; and RPA 702 5.0

Problems and Objectives

Tobacco has an annual farm value of more than \$1 billion. In addition, Federal, State and local taxes exceed \$3 billion annually. Fungi and bacteria are partly responsible for the deterioration of agricultural products in transit and storage, but very little work has been done to determine the precise environmental and cultural practices that contribute to storage losses by these organisms. No suitable method for determining the moisture content of tobacco is available for use on the sales floor. Failure to include moisture content in grade standards may result in gross inequities to the buyer or the seller. Losses to leaf tobacco and manufactured products caused by stored-tobacco insects plus the cost of control for the years 1951-1960 were estimated to be about \$13.8 million annually. By reducing losses by 33% and the cost of control by 25% through improved insect control methods, annual savings by 1980 would be about \$4.5 million assuming production remains at the present level.

Major objectives of the research are to:

1. Study the micro-organisms responsible for tobacco deterioration and changes in chemical composition.
2. Develop methods and equipment for rapid determination of moisture content of cured tobacco.
3. Determine the micro-organisms responsible for storage losses of tobacco and how they relate to the tobacco health problem by causing mycotoxicoses.
4. Reduce losses caused by stored-tobacco insects through improved insect control procedures that minimize pesticide residues.

Progress - USDA and Cooperative Program

RPA 408 - QUALITY MAINTENANCE IN MARKETING FIELD CROPS

A. Prevention of Insect Infestation

1. Biology and ecology. Cultures of all stages of the cigarette beetle exposed to 50° F. and 70% R.H. lived for 24 weeks. Others alternated at 4-week intervals between 70° F.-50% R.H. and 50° F.-70% R.H. lived for 32 weeks. Fourth-instar larvae and adults resisted continuous low temperatures longest. Control cultures at 80° F. and 70% R.H. died from a natural toxin within 24 weeks. Insect population was maximum at 12 weeks, then declined in spite of additional medium and larger containers. Eggs and first-instar larvae survived the longest. (SP-16)
2. Improved insecticidal control. Of insecticides evaluated against the adult cigarette beetle, only Accothion and Dursban were effective both as a residual and vapor toxicant.

In vacuum fumigation, acrylonitrile at 4, 6 and 8 lb./1,000 cu. ft. killed cigarette beetle larvae at depths of 5, 7, and 9 inches, respectively, inside compressed tobacco. HCN, at 4 lb., killed larvae at all depths in cases. Acrylonitrile aerated from the tobacco rapidly, whereas HCN aerated slowly.

At atmospheric pressure, 30 p.p.m. of phosphine in air killed all stages of the cigarette beetle in tobacco hogsheads. Phosphine concentrations inside the hogshead and surrounding airspace were similar. The dosage used was 12 aluminum phosphide pellets/1,000 cu. ft. for 96 hours at 80° F. and 60% R.H. At 3- and 6-pellet dosages, some 4th-instar larvae, pupae, and adults survived. (SP-16)

RPA 501 - IMPROVEMENT OF GRADES AND STANDARDS

1. Rapid Method for Measurement of Moisture in Cured Tobacco. A prototype model of an instrument to rapidly measure the moisture content of cured tobacco has been constructed and is being subjected to preliminary testing. The design is based on the relationship between moisture content of cured tobacco leaves and the difference in transmittance values of the leaves at 1.93- and 1.80-micron wavelengths. A $\frac{3}{4}$ " square section of several leaves from a large quantity of tobacco are used for the measurement. Several processors have indicated interest in the meter for use in redrying plants. (FC-50(A))

RPA 702 - PROTECT FOOD SUPPLIES FROM HARMFUL MICRO-ORGANISMS AND NATURALLY OCCURRING TOXINS

1. Microflora of Burley and Dark Air-Cured Tobacco and Their Effects on Quality. A total of 1,091 isolates of micro-organisms were obtained from 11 grades of leaves of dark air-cured tobacco stored in hogsheads for 1 to 5 years. The microflora consisted mainly of Actinomycetes, yeasts, Aspergillus flavus, and bacteria. Aflatoxins produced by seven isolates of A. flavus obtained from dark air-cured tobacco were extracted and identified by thin-layer chromatography. Fractions of extracted aflatoxins, similar to aflatoxin B₁ and G₁ standards, were fatal to developing chicken embryos at low concentrations. (FC-52(C))

2. Microflora of Flue-Cured Tobacco and Their Effects on Quality. Fungi were isolated from 100 samples of nondamaged marketed flue-cured tobacco from 12 different warehouses. Eleven genera were isolated, including ten species of Aspergillus. The fungi isolated from Middle Belt tobacco were Alternaria-40.6%, Aspergillus niger-47.8%, A. repens-38.0%, and Penicillium-25.8%. Old Belt tobacco contained Alternaria-74.0%, Penicillium-52.5%, Aspergillus repens-38.0%, and A. ruber-36.2%. The other genera isolated were Botrytis, Cladosporium, Epicoccum, Fusarium, Mucor, Nigrospora, Rhizopus, and Syncephalastrum. The mean moisture content (wet-weight) was 18.0% for Middle Belt tobacco and 21.6% for Old Belt tobacco.

Four days after inoculations with Aspergillus amstelodami, A. flavus, A. ochraceus, A. repens, A. ruber, and Penicillium sp., mature tobacco leaves were harvested, portions were cultured and the remainder flue-cured. Subsequent harvests and culturing occurred at weekly intervals for 3 additional weeks. Alternaria and Cladosporium were not used as inoculum but were isolated from 95% and 49%, respectively, of the noncured leaves. In contrast, Penicillium sp. and Aspergillus spp. were reisolated from 1.5% and 1%, respectively, of the noncured leaves. After the first two harvest dates, none of the storage fungi inoculated onto the leaves were reisolated from mature noncured leaves. A. repens was most commonly isolated from cured leaf tissue for the first two harvests regardless which of the six fungi was the inoculum. Alternaria was most commonly isolated for the last two harvests.

The fungi growing from 100 samples of damaged and nondamaged tobacco were identified. Aspergillus and Penicillium were commonly isolated from damaged and nondamaged tobacco, whereas Alternaria, Cladosporium, Fusarium, and Rhizopus were more frequently isolated from nondamaged tobacco. The fungi responsible for the damage appeared to be Aspergillus spp. and Penicillium.

The effects of temperature on growth of four genera, including six species of Aspergillus were compared. Generally the aspergilli grew better at higher temperatures than did Alternaria, Cladosporium, and Penicillium. Alternaria grew over the widest temperature range, whereas A. ruber and A. ochraceus had the narrowest range for growth. A. flavus grew better at higher temperatures (32-36° C.) and Alternaria grew better at lower temperatures (12-16° C.).

Results from a preliminary experiment with tobacco inoculated with spores of A. ochraceus and stored for 3 weeks at seven relative humidities ranging from 75-95%, indicate A. ochraceus grows in tobacco at 87% R.H. (33.7% m.c.), but not at 85% R.H. (27.8% m.c.) and below.

Studies on the pH requirements of some of the fungi isolated from moldy tobacco showed that A. ochraceus produced most mycelium at a final pH of 6.0-7.0; A. amstelodami, 3.0-5.0; A. tamarii, 6.4-8.0; Alternaria, 5.2-7.0; Epicoccum, 5.0-7.0; Penicillium 4.5-6.5; Helminthosporium, 3.0-7.0; Nigrospora, 7.0-8.0. Six thermophilic fungi were isolated from flue-cured tobacco. The most prevalent fungi found in samples of moldy tobacco from Honduras were Aspergillus and Penicillium; those from Turkey were Alternaria, Aspergillus and Penicillium. Significant numbers of bacteria, including Staphylococci, were present on redried leaf and in cigarettes. Identification of bacteria associated with tobacco is underway. (FC-51(A))

Publications - USDA and Cooperative Program

Prevention of Insect Infestation

Childs, Dana P., Overby, James E., and Watkins, Betty J. 1967. Low-temperature effect on cigarette beetle infestations in tobacco hogsheads. Bul. Ent. Soc. Amer. 13(3):205. Abst. (SP-16)

PHYSICAL AND ECONOMIC EFFICIENCY IN
MARKETING FIELD CROPS
Transportation and Facilities Research Division, ARS
RPA 504

USDA and Cooperative Program

<u>Location of Intramural Work</u>	<u>Scientist</u> <u>Man-Years FY 1968</u>
North Carolina	1.1

Problems and Objectives

Improved varieties of many field crops with their increased yields, along with progressive cultural and harvesting practices, require improved techniques and equipment for economically handling, conditioning and storing these crops in marketing channels. Grain now must be handled and conditioned in large quantities in a short period of time yet its quality must be maintained and labor and other costs minimized. Although product deterioration in storage has been reduced in recent years due to increased acceptance of aeration, shrinkage from loss of moisture is still a factor as well as loss from handling. Physical damage to the product must be reduced to maintain a high standard of quality. Because certain field crops are susceptible to contamination by toxin-producing molds, conditioning techniques and equipment must provide mold inhibiting environments.

Major objectives of this research are to:

1. Determine requirements essential to the development of improved techniques, equipment and facilities for conditioning, drying, storing and handling field crops.
2. Develop and test improved techniques and equipment for drying and storing field crops while maintaining desired standards of quality.
3. Develop and test improved techniques and equipment for the efficient handling of field crops yet minimize physical damage to the product.
4. Develop and test improved techniques and equipment to prepare field crops for market.
5. Establish design principles and improved facilities for commercial storage of field crops.

Progress - USDA and Cooperative Programs

1. Handling tobacco at auction warehouses. In cooperation with the North Carolina Agricultural Experiment Station, through research conducted at Raleigh, a system was developed for receiving tobacco at auction warehouses using a 10-man crew, with each crew member assigned specific responsibilities. The system; which uses gravity roller conveyors, costing about \$1,000; and forklifts renting for \$225 a month was installed and used commercially by Carolina Warehouse at Fuquay, and Centre Brick Warehouse at Wilson, to receive all their loose leaf tobacco during the 1967 marketing season. During the second year of operation the experimental grading and sales line at Fuquay was redesigned to eliminate storage between weighing and grading. Each operation was designed for a 6-second interval to provide the buyers with 10 baskets a minute. Thirty-six baskets were scheduled through the system each sales day with the maximum limited to 50 baskets because of length of loadout line. This experimental system was operated each sale's day between Sept. 7 and Nov. 21, for 6-10 percent of the selling time allotted to the warehouse. Fifty-seven growers sold 180,293 pounds of tobacco using the system during this period.

Publications - USDA and Cooperative Programs

GRAVES, A. H. 1968. Handling tobacco in warehouses. North Carolina State Agricultural Experiment Station. Annual Report.

ECONOMICS OF MARKETING TOBACCO
Marketing Economics Research Division, ERSUSDA and Cooperative Program

Research Problem Area	Location of Intramural Work	Scientist-Man- Years FY 1968
501 - Improvement of Grades and Standards	Tennessee	.3
509 - Marketing Firm and System Efficiency	Washington	1.5

Problems and Objectives

Market prices for many agricultural products do not adequately reflect differences in their use values. This creates a major problem in pricing efficiency. Under these conditions market prices cannot provide effective guides for production or marketing decisions. The identification and evaluation of individual factors affecting use value is critical to developing adequately descriptive grades and standards which in turn provide the means of effective communication and pricing efficiency in the system.

Major objectives of research on grades and standards are to:

1. Determine the significant factors affecting the use value of selected raw agricultural commodities and evaluate their relationship to processing performance and price.
2. Develop more accurate quantitative measures of these factors.
3. Develop a more effective pricing system for agricultural commodities on the basis of measurable and pertinent quality factors.

More than \$100 billion of consumer expenditures for agricultural products go for marketing, processing, and transportation services. The potential returns to improve efficiency are large but the adjustments within individual sectors must be coordinated with other sectors of the system to optimize results. In addition, improved measures of the performance of the marketing system and its subsectors are vital if these potential savings are to be realized.

Major objectives of the research on marketing firm and system efficiency are to:

1. Develop new measures of market performance.
2. Evaluate changing trends in marketing practices, utilization, and distribution patterns upon costs and efficiency of the individual firm and the marketing system as a whole.
3. Analyze costs and efficiencies of alternative agricultural product marketing and manufacturing systems.

Progress - USDA and Cooperative Programs

Tobacco - Grades and Standards

A model of tobacco processing was developed under contract with the Research Triangle Institute which identifies the various stages and processes involved, and outlines the flow of tobacco through the system. The model contains a considerable amount of basic engineering information pertinent to tobacco processing. However, a considerable amount of work will be required from

ERS personnel to put this model into a form useful for economic analysis. The sample design and sampling technique developed by Research Triangle Institute to collect grade data for relating Federal and commercial grades will require further refinement.

Tobacco Marketing Firm and System Efficiency

A cost study of tobacco handling, processing, storing and selected services related to storage for flue-cured, Burley, dark air-cured, fire-cured, and Maryland tobaccos is nearing completion. Data in this study were updated to reflect 1968 variable operating costs. Standardized depreciation and interest rates were used.

Average costs for redrying flue-cured tobacco, per hogshead, per month, was 23 cents in storage warehouses operating at 90 percent of capacity.

The sampling cost, which includes removing the hogshead from the tobacco, sampling, and replacing the hogshead was \$7.56. Costs of other services include innage, 76 cents; outage, 70 cents; reweight-restore, 87 cents; and reweigh while performing other services, 27 cents.

INDIVIDUAL FARM ADJUSTMENTS AND MANAGEMENT
Farm Production Economics, ERS

RPA 316

USDA and Cooperative Program

Subject	Location of Intramural Work	Scientist Man- Years FY 1968
Tobacco, Cotton and Peanut Adjustments 1/		
: Georgia	:	0.7
: Kentucky	:	2.0
: North Carolina	:	1.0
: South Carolina	:	1.0
: Tennessee	:	1.0
: Virginia	:	1.0
: Arkansas	:	0.1
: Louisiana	:	1.4
: Mississippi	:	0.4
: Missouri	:	1.0
: Oklahoma	:	3.0
: Texas	:	5.0
TOTAL	:	17.6

1/ The effort reported above is part of a total Federal effort involving 63 SMY's devoted to Research Problem Area 316. The three crops included above may be alternative crops in the same geographical area and similar farm management decisions would be applicable. Other studies in RPA 316 pertain to rice, wheat, financial management, risks and insurance, pesticide use, productivity, costs and returns.

Problems and Objectives

Farm business management research helps farmers adjust to the continuing process of technological, economic, and social change. Purchased inputs are increasingly being substituted for labor and land. New improved technology changes the competitive position of alternative production methods and systems. New ways of doing business with supply and marketing firms require new types of decisions by farm operators, and open up new sources of financing. Changing market demands require adjustments in products produced. The large investments required for today's commercial farms emphasize the necessity of sound financial management, including risk and insurance.

Individual farm adjustments and management for livestock and poultry farms are covered under Improved Livestock and Poultry Production Management Systems (RPA 313).

Adjustments made on individual farms and overall adjustments in total supply are interrelated; each influences the other. Continuing analyses of trends in farm output and productivity are needed. Also needed are continuing appraisals of aggregate production response and needs for adjustment as discussed under Government Programs to Balance Farm Output (RPA 808).

Major objectives of the research are to:

1. Determine and appraise the need for, profitability of, and obstacles to, adjustments (for other than primarily livestock farming).
2. Formulate and evaluate improved production plans or systems including size of business, combinations of enterprises, and new or improved practices and technology.
3. Identify and evaluate the elements of sound farm financial management.
4. Determine current and prospective trends in farm output and productivity.
5. Determine costs and returns on representative farms by major types of farming.

Progress - USDA and Cooperative Program

Appraisal of Adjustments in Tobacco, Cotton, and Peanut Areas

1. Another regional report soon to be published in connection with S-42 examines the supply response of tobacco in the major burley tobacco areas of Kentucky, Tennessee, and Virginia. The Analyses reported in this publication show the tobacco production, farm organization, and farm income for various allotment levels and prices of tobacco. The area results show that burley tobacco is by far the most profitable crop, and that considerable acreages would be produced even at the lowest prices evaluated.

2. In an allied research activity based in Kentucky, the location of burley tobacco production under some alternative Government control programs was analyzed. A programming matrix for nine regions of the Burley Belt was developed and the effect of tobacco prices ranging from 35 cents per pound to 90 cents per pound, and allotment levels ranging from 40 percent of the 1966 allotment to 140 percent, were evaluated in each of the nine areas. The results indicate that the Inner and Outer Bluegrass regions of Kentucky, the Western Pennyroyal area of Kentucky, and part of the Highland Rim of Tennessee have a comparative advantage in the production of burley tobacco.

3. In the Mississippi Delta a survey was made to obtain data on performance rates, size of labor force, and the number and size of tractors used on Delta farms. Tabulation of the data has been completed and the analysis is in process. The survey indicated a sharp increase in the use of 6-row farm equipment with a concomitant decrease in the quantity of labor used. The study of various herbicides and their effect on production practices and costs continued in the Mississippi Delta. Data from the 1966 and 1967 crops indicated that post-emergence weed control costs were not significantly affected by the kind of pre-emergence herbicide used. The tests using the least expensive pre-emergent herbicide had the lowest total weed control cost. The yields from these tests were equal or superior to those obtained in the tests in which more expensive pre-emergence herbicides were used.

4. In Louisiana, work has continued on the analysis of data previously collected for the purpose of developing improved input-output coefficients for the major crops in the Mississippi River Delta area. A soil moisture accounting system has been developed and used to generate estimates of days available for field work. A report is in process of publication.

Publications - USDA and Cooperative Program

Appraisal of Adjustments in Tobacco, Cotton, and Peanut

GIVAN, WILLIAM D. 1967. Evaluating total farm machinery costs. Virginia Farm Economics No. 204. pp. 5-10.

MARTIN, J. ROD and PLAXICO, JAMES S. 1967. Polyperiod analysis of growth and capital accumulation of farms in the Rolling Plains of Oklahoma and Texas. U. S. Dept. Agr. Tech. Bul. No. 1381. 104 pp.

WHERLY, JAMES S. and MARTIN, JAMES R. 1967. Economic problems and present research status of irrigation in the Southern Plains. Irrigation as a factor in the growth operation and survival of Great Plains farms. Great Plains Agricultural Council Publication No. 30, pp. 50-72.

SUPPLY, DEMAND AND PRICE ANALYSIS
Economic and Statistical Analysis, ERSUSDA and Cooperative Program

Research Problem Area	Location of Intramural Work	Scientist-Man- Years FY 1968
506 - Supply, Demand and Price Analysis	Washington, D.C.	2.0

Problems and Objectives

Farmers, processors, distributors, policy officials, and others need continuing economic intelligence regarding supply, demand, and price prospects to aid them in making sound production and marketing decisions to help assure adequate, well-balanced supplies of food and fiber. To insure that the situation and outlook work is as accurate and precise as possible, continuing research is needed on supply response to price, effect of supplies on price, and the effect of changing demand on prices and income.

Major objectives of the research are to:

1. Provide research needed for strengthening situation and outlook work.
2. Improve forecasts of future supply and demand prospects.
3. Appraise market situations and prospects to aid decision makers in making sound and profitable decisions.
4. Appraise current and proposed programs to aid officials in formulating agricultural programs and policies.
5. Develop and improve basic statistics for use in statistical and economic analysis.

Progress - USDA and Cooperative Programs

Changes in the tobacco situation in recent years have made it necessary to give increasing attention to the factors affecting overall tobacco use, both domestic and export. Particularly intensive analysis was made of domestic use of flue-cured tobacco and the reconciliation with the usual calculated disappearance and manufacturers' reported disappearance. A major factor in the 9 percent drop in flue-cured domestic disappearance during 1966/67 was the sharp increase in use of reconstituted tobacco sheet (as indicated by stock changes). Further stress was placed in the June and September issues of the Tobacco Situation on the impact on imports and exports of larger overseas supplies from nontraditional suppliers. The relatively high price of U.S. tobacco along with changes in manufacturing techniques have slowed down the rate of utilization of U. S. tobacco. The September Tobacco Situation analyzes the changes in the outlook for tobacco because of the 2 percent downturn in per capita cigarette consumption. Some users are apparently reacting to the smoking-health publicity. The June issue of the Situation carried an analysis of commercial tobacco farms based on the 1964 Census of Agriculture.

Publications - USDA and Cooperative Program

SACKRIN, S. M. and (beginning June 1968) MILLER, ROBERT H. Tobacco Situation, published quarterly. ERS, USDA, Washington, D. C.

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MILLER, ROBERT H. July 22, 1968. "Estimating Domestic Tobacco Use Through Regression Methods." Paper presented at the 22nd National Tobacco Workers Conference at Asheville, North Carolina. 12 pp.